INTERNATIONAL SEARCH REPORT

[redacted]ational Application No
[redacted]/CA 00/00642

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12N15/29 C12N15/82 C12N5/10 C07K14/415 A01H5/00
A01H5/10

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C12N C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
CHEM ABS Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	NAKAMURA Y.: "AC AB025629" EMBL DATABASE, 9 April 1999 (1999-04-09), XP002120887 Heidelberg the whole document ---	1
X	ELLIOTT R. ET AL.: "AINTEGUMENTA, an APETALA2-like gene of arabidopsis with pleiotropic roles in ovule development and floran organ growth" THE PLANT CELL, vol. 8, 1996, pages 155-168, XP002120889 cited in the application * see esp. p.161;165 * --- -/--	54

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
10 October 2000	03/11/2000

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Kania, T

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
X	DATABASE EBI 'Online! AC AL008970, 18 November 1997 (1997-11-18) HAMLIN N. ET AL.: "Plasmodium falciparum MAL3P4" XP002149645 abstract	63
A	RIEGER M. ET AL.: "AC AL049862" EMBL DATABASE, 13 May 1999 (1999-05-13), XP002120888 Heidelberg the whole document	28,29
A	WO 97 43427 A (CIBA GEIGY AG ;VRIES SAPE CORNELIS DE (NL); SCHMIDT EDUARD DANIEL) 20 November 1997 (1997-11-20) cited in the application the whole document	1-62
A	WO 98 37184 A (UNIV CALIFORNIA) 27 August 1998 (1998-08-27) * see esp. example 2 *	1-62
A	WO 98 07842 A (JOFUKU K DIANE ;OKAMURO JACK K (US); UNIV CALIFORNIA (US)) 26 February 1998 (1998-02-26) the whole document	1-62
A	OGAS J. ET AL.: "Cellular differentiation regulated by gibberellin in the Arabidopsis thaliana pickle mutant" SCIENCE, vol. 277, July 1997 (1997-07), pages 91-94, XP002120890 cited in the application the whole document	1-62
A	BOUTILIER, K. A. ET AL: "Expression of the BnmNAP subfamily of napin genes coincides with the induction of Brassica microspore embryogenesis" PLANT MOLECULAR BIOLOGY, (1994) VOL. 26, NO. 6, PP. 1711-1723. 48 REF. ISSN: 0167-4412, XP002120891 cited in the application the whole document	1-62

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
A	CUSTERS, JAN B.M. ET AL: "Regulation of the inductive phase of microspore embryogenesis in Brassica napus" ACTA HORTIC. (1996), 407(INTERNATIONAL SYMPOSIUM ON BRASSICAS, NINTH CRUCIFER GENETICS WORKSHOP, 1994), 209-217, XP002120892 * see the whole document; esp. table 3 clone 13 * ---	1-62
A	CHAUDHURY A. ET AL.: "Ovule and embryo development, apomixis and fertilization" CURRENT OPINION IN PLANT BIOLOGY, 'Online! vol. 1, 1998, pages 26-31, XP002120893 Retrieved from the Internet: <URL:http://biomednet.com/elecref/1369526600100026> 'retrieved on 1999-10-27! * see esp. p.29 * -----	1-62

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INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/CA 00/00642

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9743427 A	20-11-1997	AU 713130 B	25-11-1999
		AU 2953997 A	05-12-1997
		BR 9709098 A	03-08-1999
		CA 2254839 A	20-11-1997
		CN 1218510 A	02-06-1999
		EP 0915984 A	19-05-1999
		HU 9901477 A	28-09-1999
		PL 329872 A	12-04-1999
WO 9837184 A	27-08-1998	AU 6328398 A	09-09-1998
		EP 0977836 A	09-02-2000
WO 9807842 A	26-02-1998	US 5994622 A	30-11-1999
		AU 3986797 A	06-03-1998
		EP 0964920 A	22-12-1999
		PL 331771 A	02-08-1999
		US 6093874 A	25-07-2000

XR

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference	FOR FURTHER ACTION	see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.
08-887547WO		
International application No.	International filing date *(day/month/year)*	(Earliest) Priority Date *(day/month/year)*
PCT/CA 00/ 00642	02/06/2000	02/06/1999

Applicant

PLANT RESEARCH INTERNATIONAL et al.

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 4 sheets.

[X] It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**

a. With regard to the **language,** the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

[ ] the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

b. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international search was carried out on the basis of the sequence listing :

[X] contained in the international application in written form.

[ ] filed together with the international application in computer readable form.

[ ] furnished subsequently to this Authority in written form.

[X] furnished subsequently to this Authority in computer readble form.

[X] the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

[X] the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

2. [ ] **Certain claims were found unsearchable** (See Box I).

3. [ ] **Unity of invention is lacking** (see Box II).

4. With regard to the **title,**

[ ] the text is approved as submitted by the applicant.

[X] the text has been established by this Authority to read as follows:

USE OF THE BNM3 TRANSCRIPTIONAL ACTIVATOR TO CONTROL PLANT EMBRYOGENESIS AND REGENERATION PROCESSES

5. With regard to the **abstract,**

[X] the text is approved as submitted by the applicant.

[ ] the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is Figure No. 1

[X] as suggested by the applicant.

[ ] None of the figures.

[ ] because the applicant failed to suggest a figure.

[ ] because this figure better characterizes the invention.

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
14 December 2000 (14.12.2000)

PCT

(10) International Publication Number
WO 00/75330 A1

(51) International Patent Classification[7]: C12N 15/29, 15/82, 5/10, C07K 14/415, A01H 5/00, 5/10

(21) International Application Number: PCT/CA00/00642

(22) International Filing Date: 2 June 2000 (02.06.2000)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
99201745.9 2 June 1999 (02.06.1999) EP

(71) Applicants *(for all designated States except US)*: **PLANT RESEARCH INTERNATIONAL** [NL/NL]; Building No. 107, Droevendaalsesteeg 1, NL-6708 PB Wageningen (NL). **HER MAJESTY THE QUEEN IN RIGHT OF CANADA, as represented by THE MINISTER OF AGRICULTURE AND AGRI-FOOD CANADA** [CA/CA]; Eastern Cereal & Oilseed Research Centre, K.W. Neatby Building, Ottawa, Ontario K1A 0C6 (CA).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **BOUTILER, Kim** [CA/NL]; Groen van Prinstererstraat 87, NL-6702 CP Wageningen (NL). **OUELLET, Thérèse** [CA/CA]; 115 Kennevale Drive, Nepean, Ontario K2J 3X7 (CA). **CUSTERS, Jan** [NL/NL]; Gen. Foulkesweg 25, NL-6703 BL Wageningen (NL). **HATTORI, Jiro** [JP/CA]; 763 Halstead Street, Ottawa, Ontario K1G 1M5 (CA). **MIKI, Brian** [CA/CA]; 1876 Dorset Drive, Ottawa, Ontario K1H 5V1 (CA). **VAN LOOKEREN CAMPAGNE, Michiel** [NL/BE]; Lievestraat 31, B-9920 Lovendegem (BE).

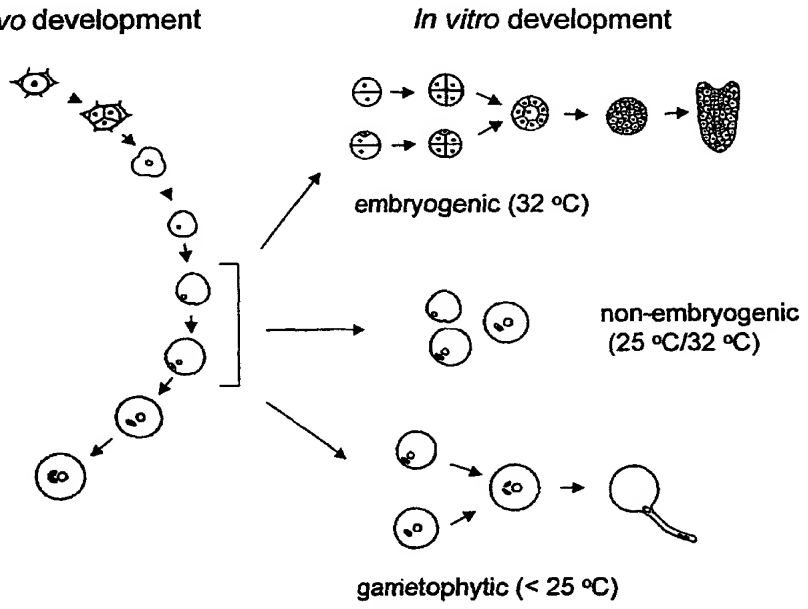
(74) Agents: **SECHLEY, Konrad, A.** et al.; Gowling Lafleur Henderson LLP, Suite 2600, 160 Elgin Street, Ottawa, Ontario K1P 1C3 (CA).

(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian

[Continued on next page]

(54) Title: USE OF THE BNM3 TRANSCRIPTIONAL ACTIVATOR TO CONTROL PLANT EMBRYOGENESIS AND REGENERATION PROCESSES



(57) Abstract: The present invention provides for a gene obtained during the induction of microspore embryogenesis. The protein encoded by this gene renders plant cells embryogenic, and increases the regenerative capacity of the plant cell. Also disclosed is the regulatory region of this gene and its use for directing the expression of a gene of interest within a suitable host cell.

WO 00/75330 A1

patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published:

— *With international search report.*

— *Before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments.*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

INTERNATIONAL SEARCH REPORT

Intern[illegible]plication No
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	-/--	

[X] Further documents are listed in the continuation of box C.

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° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

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"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
10 October 2000	03/11/2000

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Kania, T

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
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A	WO 98 37184 A (UNIV CALIFORNIA) 27 August 1998 (1998-08-27) * see esp. example 2 *	1-62
A	WO 98 07842 A (JOFUKU K DIANE ;OKAMURO JACK K (US); UNIV CALIFORNIA (US)) 26 February 1998 (1998-02-26) the whole document	1-62
A	OGAS J. ET AL.: "Cellular differentiation regulated by gibberellin in the Arabidopsis thaliana pickle mutant" SCIENCE, vol. 277, July 1997 (1997-07), pages 91-94, XP002120890 cited in the application the whole document	1-62
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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

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1

INTERNATIONAL SEARCH REPORT

...mation on patent family members

Intern...cation No
PCT/CA 00/00642

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9743427 A	20-11-1997	AU 713130 B	25-11-1999
		AU 2953997 A	05-12-1997
		BR 9709098 A	03-08-1999
		CA 2254839 A	20-11-1997
		CN 1218510 A	02-06-1999
		EP 0915984 A	19-05-1999
		HU 9901477 A	28-09-1999
		PL 329872 A	12-04-1999
WO 9837184 A	27-08-1998	AU 6328398 A	09-09-1998
		EP 0977836 A	09-02-2000
WO 9807842 A	26-02-1998	US 5994622 A	30-11-1999
		AU 3986797 A	06-03-1998
		EP 0964920 A	22-12-1999
		PL 331771 A	02-08-1999
		US 6093874 A	25-07-2000

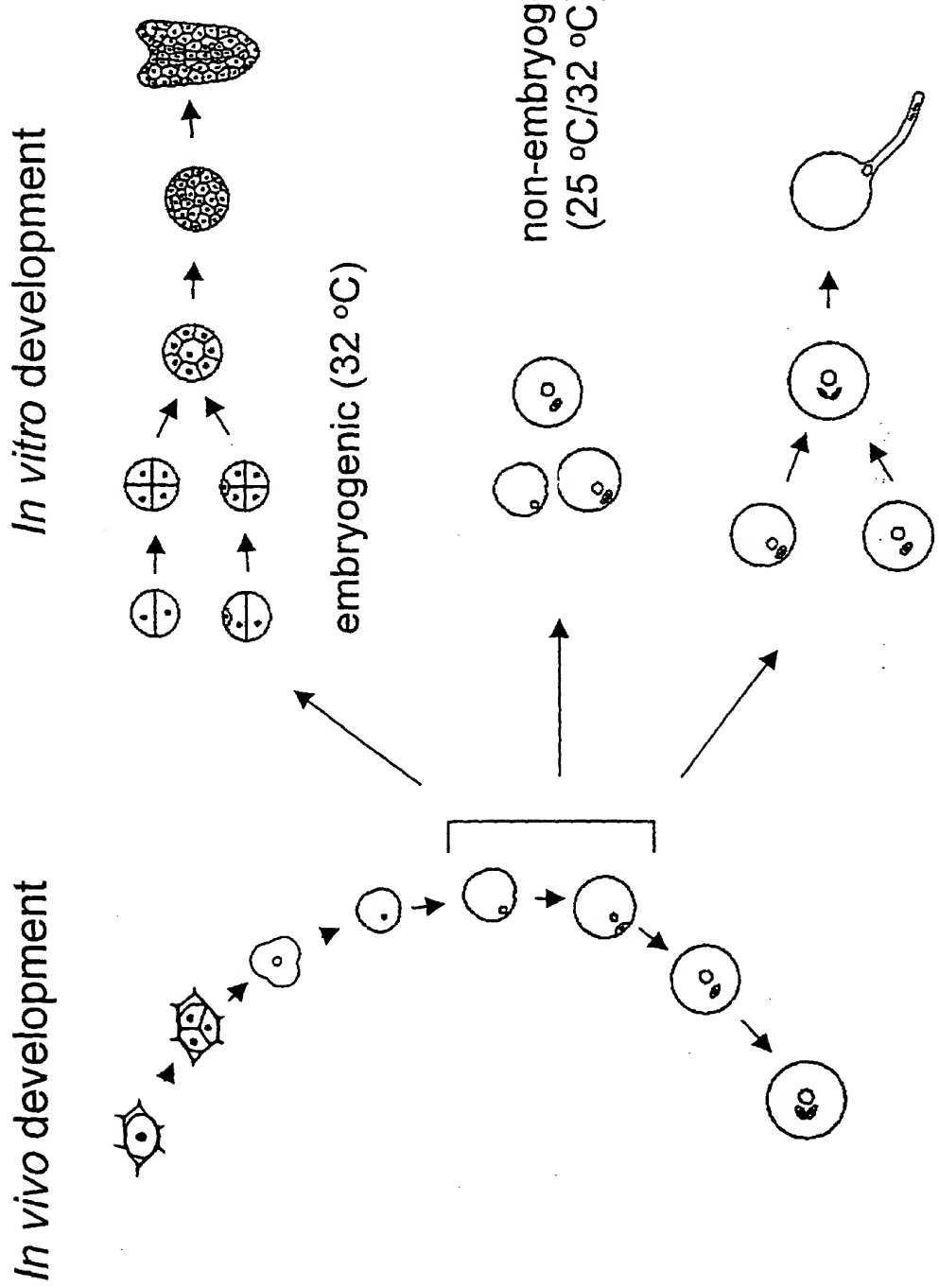
In vivo development
In vitro development
embryogenic (32 °C)
non-embryogenic (25 °C/32 °C)
gametophytic (< 25 °C)

FIGURE 1

FIGURE 2

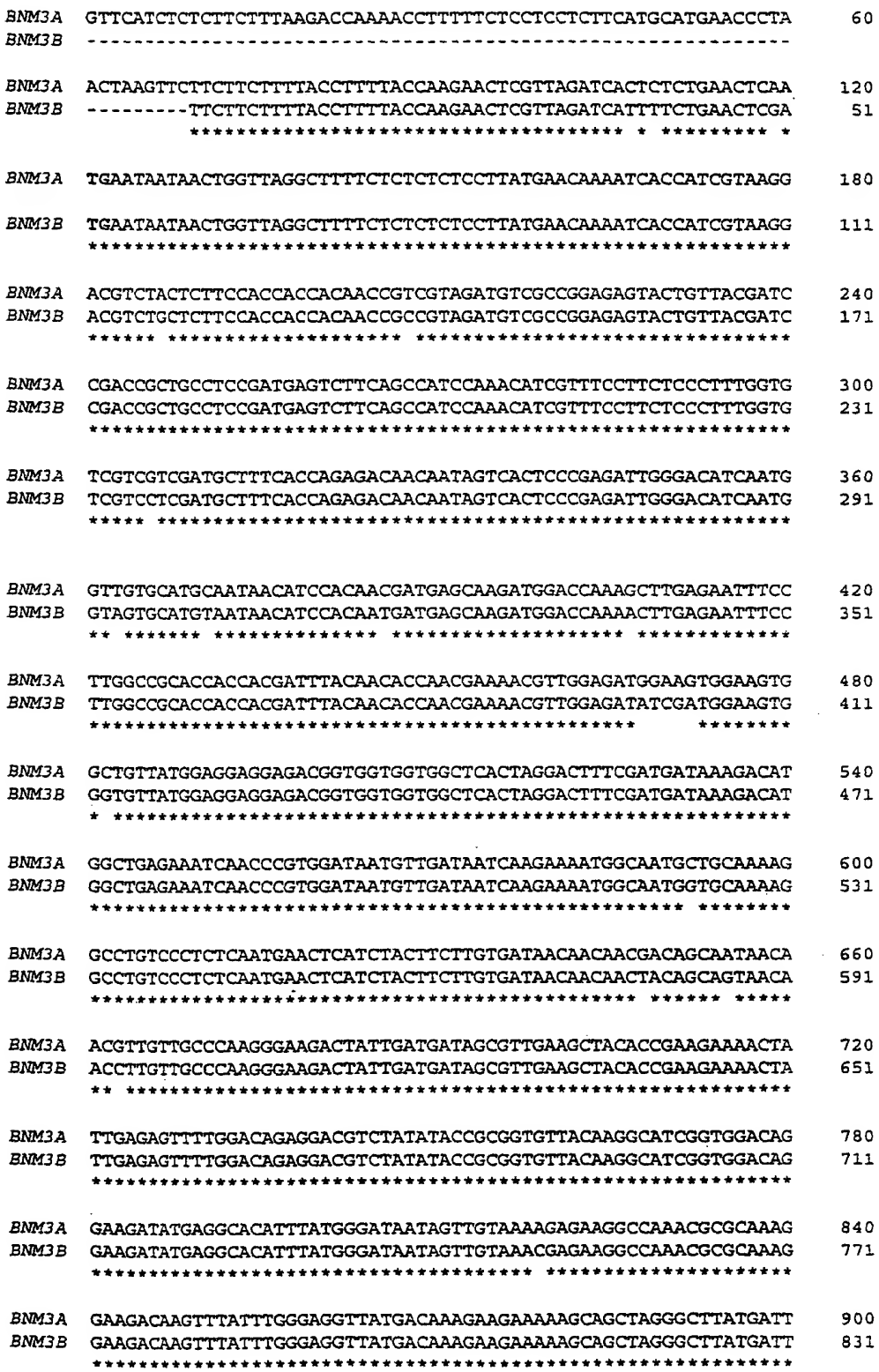
BNM3A GTTCATCTCTCTTCTTTAAGACCAAAACCTTTTTCTCCTCCTCTTCATGCATGAACCCTA 60
BNM3B ----------
BNM3A ACTAAGTTCTTCTTCTTTTACCTTTTACCAAGAACTCGTTAGATCACTCTCTGAACTCAA 120
BNM3B ---------TTCTTCTTTTACCTTTTACCAAGAACTCGTTAGATCATTTTCTGAACTCGA 51
BNM3A TGAATAATAACTGGTTAGGCTTTTCTCTCTCTCCTTATGAACAAAATCACCATCGTAAGG 180
BNM3B TGAATAATAACTGGTTAGGCTTTTCTCTCTCTCCTTATGAACAAAATCACCATCGTAAGG 111
BNM3A ACGTCTACTCTTCCACCACCACAACCGTCGTAGATGTCGCCGGAGAGTACTGTTACGATC 240
BNM3B ACGTCTGCTCTTCCACCACCACAACCGCCGTAGATGTCGCCGGAGAGTACTGTTACGATC 171
BNM3A CGACCGCTGCCTCCGATGAGTCTTCAGCCATCCAAACATCGTTTCCTTCTCCCTTTGGTG 300
BNM3B CGACCGCTGCCTCCGATGAGTCTTCAGCCATCCAAACATCGTTTCCTTCTCCCTTTGGTG 231
BNM3A TCGTCGTCGATGCTTTCACCAGAGACAACAATAGTCACTCCCGAGATTGGGACATCAATG 360
BNM3B TCGTCCTCGATGCTTTCACCAGAGACAACAATAGTCACTCCCGAGATTGGGACATCAATG 291
BNM3A GTTGTGCATGCAATAACATCCACAACGATGAGCAAGATGGACCAAAGCTTGAGAATTTCC 420
BNM3B GTAGTGCATGTAATAACATCCACAATGATGAGCAAGATGGACCAAAACTTGAGAATTTCC 351
BNM3A TTGGCCGCACCACCACGATTTACAACACCAACGAAAACGTTGGAGATGGAAGTGGAAGTG 480
BNM3B TTGGCCGCACCACCACGATTTACAACACCAACGAAAACGTTGGAGATATCGATGGAAGTG 411
BNM3A GCTGTTATGGAGGAGGAGACGGTGGTGGTGGCTCACTAGGACTTTCGATGATAAAGACAT 540
BNM3B GGTGTTATGGAGGAGGAGACGGTGGTGGTGGCTCACTAGGACTTTCGATGATAAAGACAT 471
BNM3A GGCTGAGAAATCAACCCGTGGATAATGTTGATAATCAAGAAAATGGCAATGCTGCAAAAG 600
BNM3B GGCTGAGAAATCAACCCGTGGATAATGTTGATAATCAAGAAAATGGCAATGGTGCAAAAG 531
BNM3A GCCTGTCCCTCTCAATGAACTCATCTACTTCTTGTGATAACAACAACGACAGCAATAACA 660
BNM3B GCCTGTCCCTCTCAATGAACTCATCTACTTCTTGTGATAACAACAACTACAGCAGTAACA 591
BNM3A ACGTTGTTGCCCAAGGGAAGACTATTGATGATAGCGTTGAAGCTACACCGAAGAAAACTA 720
BNM3B ACCTTGTTGCCCAAGGGAAGACTATTGATGATAGCGTTGAAGCTACACCGAAGAAAACTA 651
BNM3A TTGAGAGTTTTGGACAGAGGACGTCTATATACCGCGGTGTTACAAGGCATCGGTGGACAG 780
BNM3B TTGAGAGTTTTGGACAGAGGACGTCTATATACCGCGGTGTTACAAGGCATCGGTGGACAG 711
BNM3A GAAGATATGAGGCACATTTATGGGATAATAGTTGTAAAAGAGAAGGCCAAACGCGCAAAG 840
BNM3B GAAGATATGAGGCACATTTATGGGATAATAGTTGTAAACGAGAAGGCCAAACGCGCAAAG 771
BNM3A GAAGACAAGTTTATTTGGGAGGTTATGACAAAGAAGAAAAAGCAGCTAGGGCTTATGATT 900
BNM3B GAAGACAAGTTTATTTGGGAGGTTATGACAAAGAAGAAAAAGCAGCTAGGGCTTATGATT 831

FIGURE 2(Cont'd)

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BNM3A  TAGCCGCACTCAAGTATTGGGGAACCACCACTACTACTAACTTCCCCATGAGCGAATATG   960
BNM3B  TAGCCGCACTCAAGTATTGGGGAACCACCACTACTACTAACTTCCCCATGAGCGAATATG   891
       ************************************************************

BNM3A  AAAAAGAGGTAGAAGAGATGAAGCACATGACAAGGCAAGAGTATGTTGCCTCACTGCGCA  1020
BNM3B  AGAAAGAGATAGAAGAGATGAAGCACATGACAAGGCAAGAGTATGTTGCCTCACTTCGCA   951
       * ****** ********************************************** ****

BNM3A  GGAAAAGTAGTGGTTTCTCTCGTGGTGCATCGATTTATCGTGGAGTAACAAGACATCACC  1080
BNM3B  GGAAAAGTAGTGGTTTCTCTCGTGGTGCATCGATTTATCGTGGAGTAACAAGACATCACC  1011
       ************************************************************

BNM3A  AACATGGAAGATGGCAAGCTAGGATAGGAAGAGTCGCCGGTAACAAAGACCTCTACTTGG  1140
BNM3B  AACATGGAAGATGGCAAGCTAGGATAGGAAGAGTCGCCGGTAACAAAGACCTCTACTTGG  1071
       ************************************************************

BNM3A  GAACTTTTGGCACACAAGAAGAAGCTGCAGAGGCATACGACATTGCGGCCATCAAATTCA  1200
BNM3B  GAACTTTTGGCACACAAGAAGAAGCTGCAGAGGCATACGACATTGCGGCCATCAAATTCA  1131
       ************************************************************

BNM3A  GAGGATTAACCGCAGTGACTAACTTCGACATGAACAGATACAACGTTAAAGCAATCCTCG  1260
BNM3B  GAGGATTAACCGCAGTGACTAACTTCGACATGAACAGATACAACGTTAAAGCAATCCTCG  1191
       ************************************************************

BNM3A  AAAGCCCTAGTCTTCCTATTGGTAGCGCCGCAAAACGTCTCAAGGAGGCTAACCGTCCGG  1320
BNM3B  AAAGCCCTAGTCTTCCTATTGGTAGCGCCGCAAAACGTCTCAAGGAGGCTAACCGTCCGG  1251
       ************************************************************

BNM3A  TTCCAAGTATGATGATGATCAGTAATAACGTTTCAGAGAGTGAGAATAGTGCTAGCGGTT  1380
BNM3B  TTCCAAGTATGATGATGATCAGTAATAACGTTTCAGAGAGTGAGAATAATGCTAGCGGTT  1311
       ************************************************** ***********

BNM3A  GGCAAAACGCTGCGGTTCAGCATCATCAGGGAGTAGATTTGAGCTTATTGCACCAACATC  1440
BNM3B  GGCAAAACGCTGCGGTTCAGCATCATCAGGGAGTAGATTTGAGCTTATTGCAGCAACATC  1371
       ************************************************** *******

BNM3A  AAGAGAGGTACAATGGTTATTATTACAATGGAGGAAACTTGTCTTCGGAGAGTGCTAGGG  1500
BNM3B  AAGAGAGGTACAATGGTTATTATTACAATGGAGGAAACTTGTCTTCGGAGAGTGCTAGGG  1431
       ************************************************************

BNM3A  CTTGTTTCAAACAAGAGGATGATCAACACCATTTCTTGAGCAACACGCAGAGCCTCATGA  1560
BNM3B  CTTGTTTCAAACAAGAGGATGATCAACACCATTTCTTGAGCAACACGCAGAGCCTCATGA  1491
       ************************************************************

BNM3A  CTAATATCGATCATCAAAGTTCTGTTTCGGATGATTCGGTTACTGTTTGTGGAAATGTTG  1620
BNM3B  CTAATATCGATCATCAAAGTTCTGTTTCAGATGATTCGGTTACTGTTTGTGGAAATGTTG  1551
       ************************** ***************************************

BNM3A  TTGGTTATGGTGGTTATCAAGGATTTGCAGCCCCGGTTAACTGCGATGCCTACGCTGCTA  1680
BNM3B  TTGGTTATGGTGGTTATCAAGGATTTGCAGCCCCGGTTAACTGCGATGCCTACGCTGCTA  1611
       ************************************************************

BNM3A  GTGAGTTTGATTATAACGCAAGAAACCATTATTACTTTGCTCAGCAGCAGCAGACCCAGC  1740
BNM3B  GTGAGTTTGACTATAACGCAAGAAACCATTATTACTTTGCTCAGCAGCAGCAGACCCAGC  1671
       ********** ***************************************************

BNM3A  AGTCGCCAGGTGGAGATTTTCCCGCGGCAATGACGAATAATGTTGGCTCTAATATGTATT  1800
BNM3B  ATTCGCCAGGAGGAGATTTTCCCGCGGCAATGACGAATAATGTTGGCTCTAATATGTATT  1731
       * ******** ***************************************************

BNM3A  ACCATGGGGAAGGTGGTGGAGAAGTTGCTCCAACATTTACAGTTTGGAACGACAATTAGA  1860
BNM3B  ACCATGGGGAAGGTGGTGGAGAAGTTGCTCCAACATTTACAGTTTGGAACGACAATTAGA  1791
       ************************************************************
```

FIGURE 2(Cont'd)

```
BNM3A  AAAAATAGTTAAAGATCTTTAGTTATATGCGTTGTTGTGTGCTGGTGAACAGTGTGATAC  1920
BNM3B  AATAATAGTTAAAGATCTTTAGTTATATGCGTTGTTGTGTGGTGTTGAACAGTTTGATAC  1851
       ** ************************************* ** ******** ******

BNM3A  TTTGATTATGTTTTTTTCTTTCTCTTTTTCTTTTTCTTGGTTAATTTCTTAAGACTTATT  1980
BNM3B  TTTGATTATGTTTTTT--TTTCTCTTTTTCATTTTGTTGGTTAGTTTCTTAAGACTTATT  1909
       ****************  ************ **** ******* ****************

BNM3A  TTTAGTTTCCATTAGTTGGATAAATTTTCAGACT-------------------------  2014
BNM3B  TTTTGTTTCCATTAGTTGGATAAATTTTCGGACTTAAGGGTCACTTCTGTTCTGACTTCT  1969
       *** ************************* ****

BNM3A  ----------------------
BNM3B  GTCTAATACAGAAAAGTTTTCAT                                        1992
```

FIGURE 3

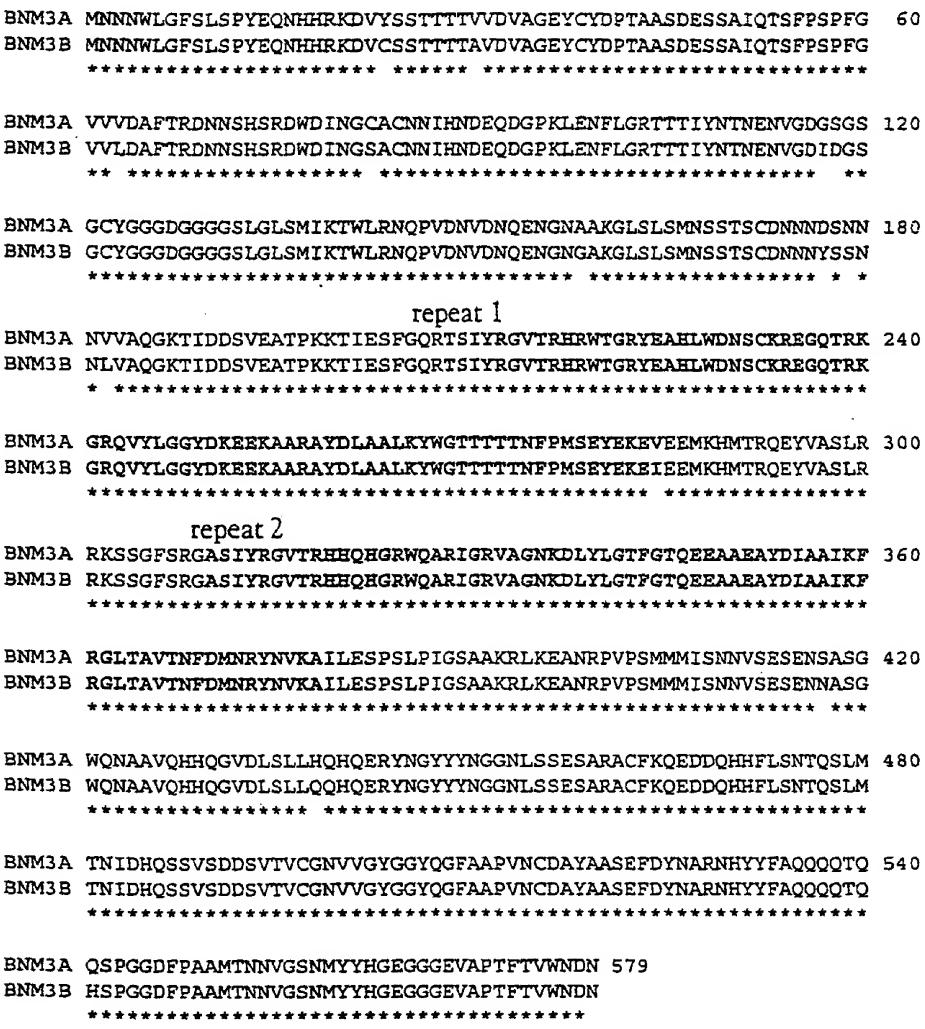

BNM3A MNNNWLGFSLSPYEQNHHRKDVYSSTTTTVVDVAGEYCYDPTAASDESSAIQTSFPSPFG 60
BNM3B MNNNWLGFSLSPYEQNHHRKDVCSSTTTTAVDVAGEYCYDPTAASDESSAIQTSFPSPFG
BNM3A VVVDAFTRDNNSHSRDWDINGCACNNIHNDEQDGPKLENFLGRTTTIYNTNENVGDGSGS 120
BNM3B VVLDAFTRDNNSHSRDWDINGSACNNIHNDEQDGPKLENFLGRTTTIYNTNENVGDIDGS
BNM3A GCYGGGDGGGGSLGLSMIKTWLRNQPVDNVDNQENGNAAKGLSLSMNSSTSCDNNNDSNN 180
BNM3B GCYGGGDGGGGSLGLSMIKTWLRNQPVDNVDNQENGNGAKGLSLSMNSSTSCDNNNYSSN
repeat 1
BNM3A NVVAQGKTIDDSVEATPKKTIESFGQRTSIYRGVTRHRWTGRYEAHLWDNSCKREGQTRK 240
BNM3B NLVAQGKTIDDSVEATPKKTIESFGQRTSIYRGVTRHRWTGRYEAHLWDNSCKREGQTRK
BNM3A GRQVYLGGYDKEEKAARAYDLAALKYWGTTTTTNFPMSEYEKEVEEMKHMTRQEYVASLR 300
BNM3B GRQVYLGGYDKEEKAARAYDLAALKYWGTTTTTNFPMSEYEKEIEEMKHMTRQEYVASLR
repeat 2
BNM3A RKSSGFSRGASIYRGVTRHHQHGRWQARIGRVAGNKDLYLGTFGTQEEAAEAYDIAAIKF 360
BNM3B RKSSGFSRGASIYRGVTRHHQHGRWQARIGRVAGNKDLYLGTFGTQEEAAEAYDIAAIKF
BNM3A RGLTAVTNFDMNRYNVKAILESPSLPIGSAAKRLKEANRPVPSMMMISNNVSESENSASG 420
BNM3B RGLTAVTNFDMNRYNVKAILESPSLPIGSAAKRLKEANRPVPSMMMISNNVSESENNASG
BNM3A WQNAAVQHHQGVDLSLLHQHQERYNGYYYNGGNLSSESARACFKQEDDQHHFLSNTQSLM 480
BNM3B WQNAAVQHHQGVDLSLLQQHQERYNGYYYNGGNLSSESARACFKQEDDQHHFLSNTQSLM
BNM3A TNIDHQSSVSDDSVTVCGNVVGYGGYQGFAAPVNCDAYAASEFDYNARNHYYFAQQQQTQ 540
BNM3B TNIDHQSSVSDDSVTVCGNVVGYGGYQGFAAPVNCDAYAASEFDYNARNHYYFAQQQQTQ
BNM3A QSPGGDFPAAMTNNVGSNMYYHGEGGGEVAPTFTVWNDN 579
BNM3B HSPGGDFPAAMTNNVGSNMYYHGEGGGEVAPTFTVWNDN

Figure 4

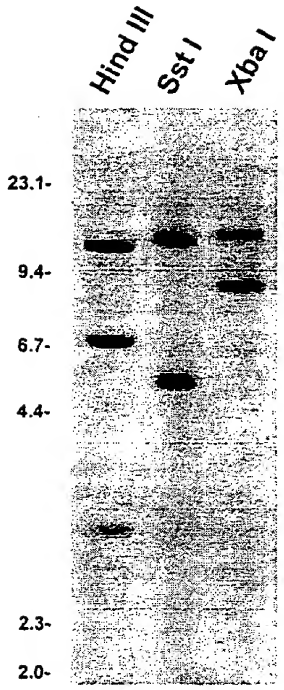


FIGURE 5

AP2 DOMAIN REPEAT 1

```
BNM3  TSIYRGVTRHRWTGRYEAHLWDNSCKREGQTRKGRQVYLGGYDKEEKAARAYDLAALKYWGTTTTTNFPMSEYEKEV
ANT   T*Q**********************F*K**HS***********M*****************PS*H***SAEN*Q**I
ZM    T*******************E***R****S*************************F**P******QV*N****L
GL15  S*Q*****FY*R***W*S*I**--C--------*K******F*TAQA******Q**I*FR*LNADI**TLDD*KD*M
AP2   S*Q*****FY*R***W*S*I**--C--------*K******F*TAHA******R**I*FR*VEADI**NIDD*DDDL
```

LINKER

```
BNM3  EEMKHMTRQEYVASLRRKSSGFSRG
ANT   *D**N********H***********
ZM    ****S*****FI*************
GL15  KK**DLSKE*F*LV***QGA**V**
AP2   KQ*TNL*KE*F*HV***Q*T**P**
```

AP2 DOMAIN REPEAT 2

```
BNM3  ASIYRGVTRHHQHGRWQARIGRVAGNKDLYLGTFGTQEEAAEAYDIAAIKFRGLTAVTNFDMNRYNVKA
ANT   ****************************************V*******TN******IT**D*DR
ZM    ********************S***********S******************N****L**S**D*ES
GL15  S*RF****-Q*KC*K*E****QLM*K*YV***LYD*ET***Q***K****CY*KE******AQS*DKEL
AP2   S*K*****-L*KC***E**M*QFL*K*YV***L*D*EV***R***K****CN*KD******PSI*DEEL
```

FIGURE 6

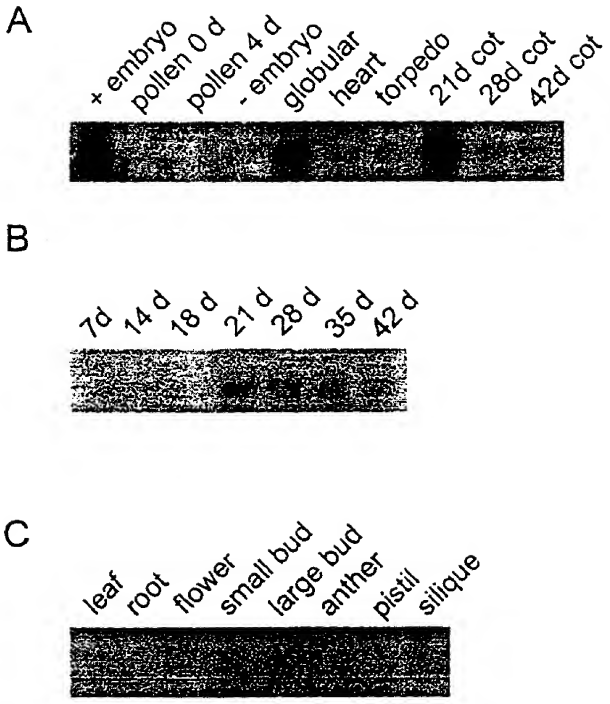
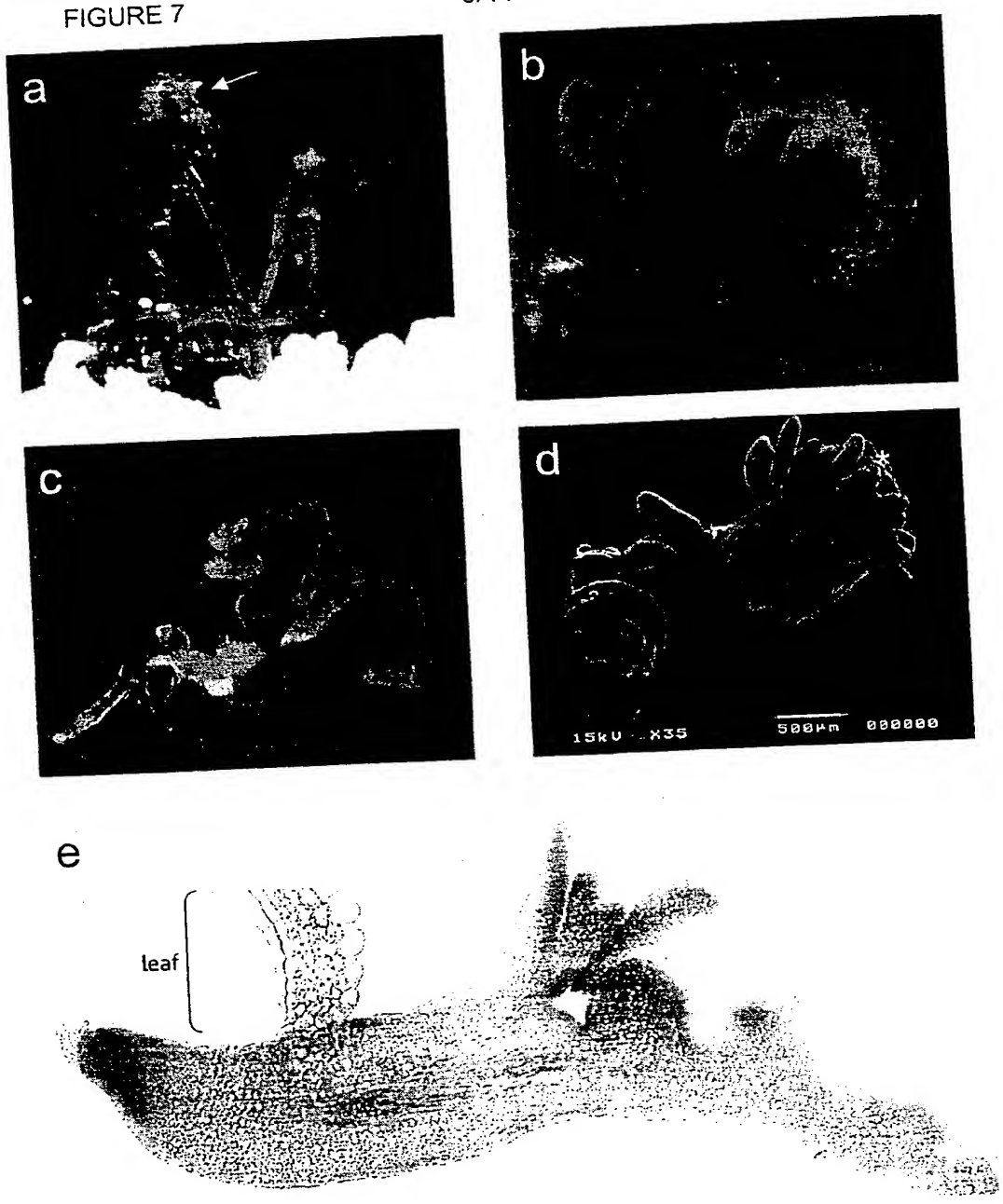

A
+ embryo
pollen 0 d
pollen 4 d
- embryo
globular
heart
torpedo
21d cot
28d cot
42d cot
B
7d
14 d
18 d
21 d
28 d
35 d
42 d
C
leaf
root
flower
small bud
large bud
anther
pistil
silique

FIGURE 7



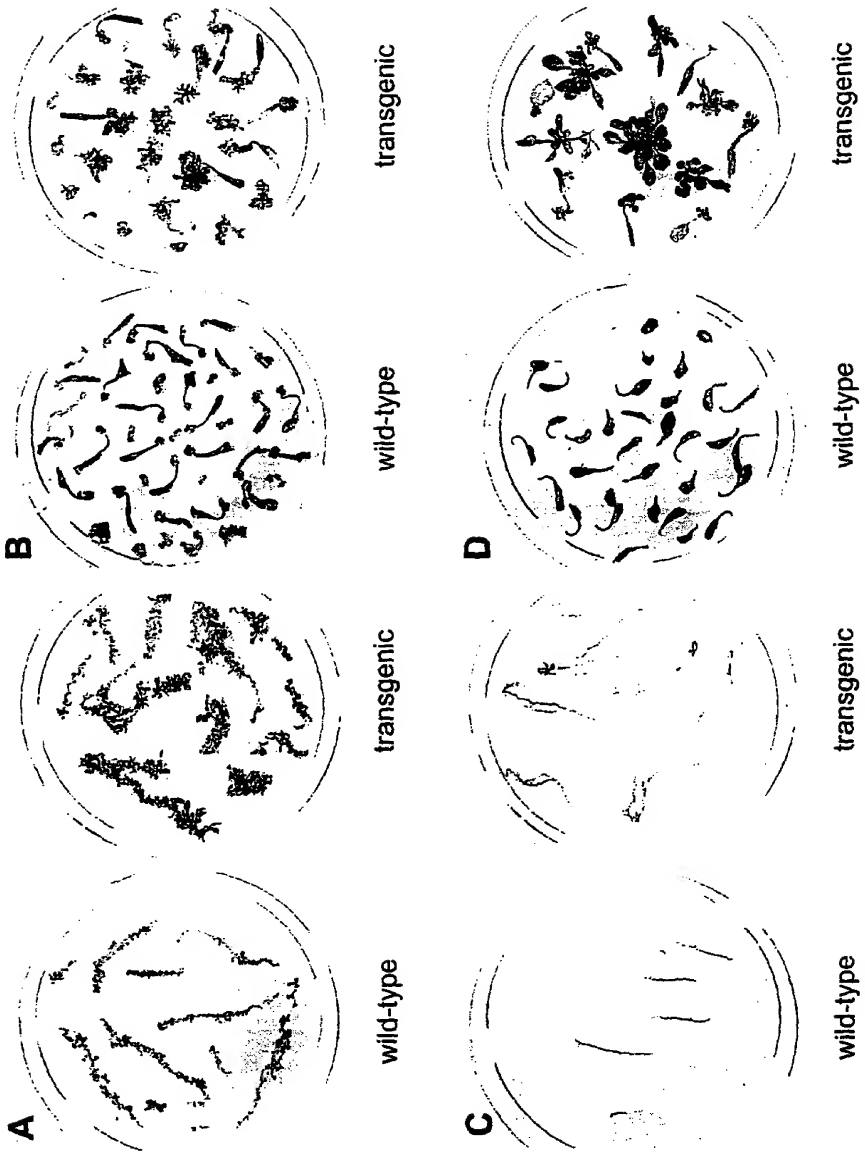
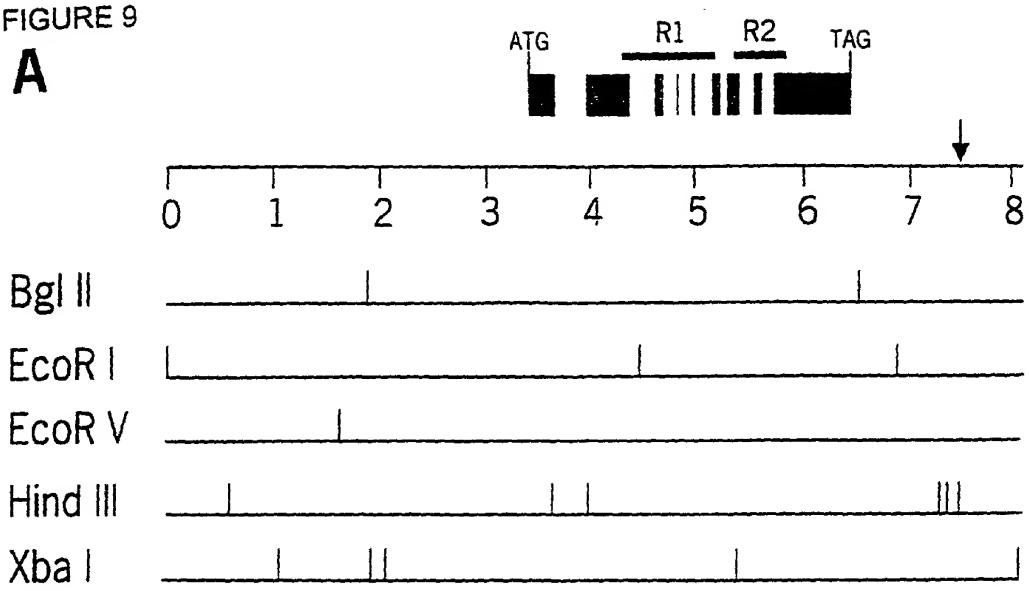

A
wild-type
transgenic
B
wild-type
transgenic
C
wild-type
transgenic
D
wild-type
transgenic

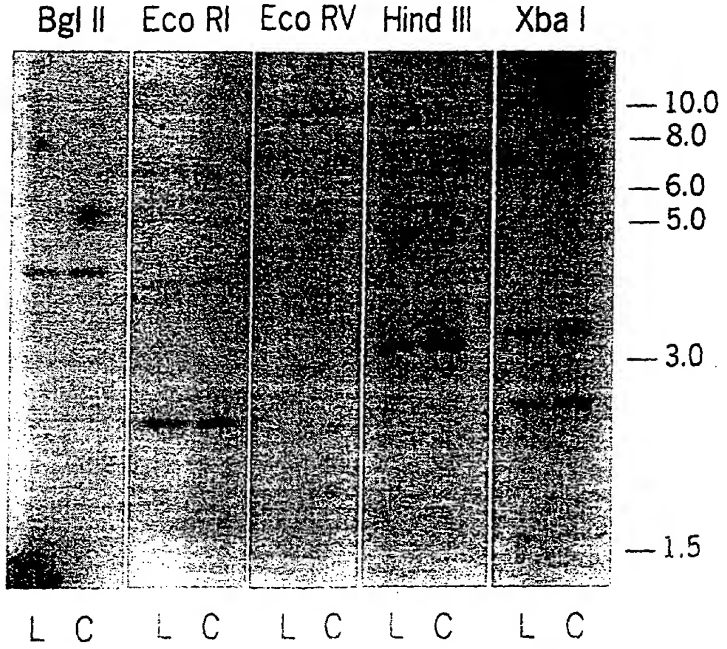
FIGURE 8

FIGURE 9

A


ATG
R1
R2
TAG
0
1
2
3
4
5
6
7
8
Bgl II
EcoR I
EcoR V
Hind III
Xba I

B


Bgl II
Eco RI
Eco RV
Hind III
Xba I
10.0
8.0
6.0
5.0
3.0
1.5
L C L C L C L C L C

PATENT COOPERATION TREATY

From the INTERNATIONAL SEARCHING AUTHORITY

PCT

NOTIFICATION OF TRANSMITTAL OF THE INTERNATIONAL SEARCH REPORT OR THE DECLARATION

(PCT Rule 44.1)

To:
Gowling Lafleur Henderson LLP
Attn. Sechley, K.
Suite 2600
160 Elgin Street
Ottawa, Ontario K1P 1C3
CANADA

Date of mailing *(day/month/year)*	03/11/2000
Applicant's or agent's file reference 08-887547WO	**FOR FURTHER ACTION** See paragraphs 1 and 4 below
International application No. PCT/CA 00/ 00642	International filing date *(day/month/year)* 02/06/2000
Applicant PLANT RESEARCH INTERNATIONAL et al.	

1. [X] The applicant is hereby notified that the International Search Report has been established and is transmitted herewith.

Filing of amendments and statement under Article 19:
The applicant is entitled, if he so wishes, to amend the claims of the International Application (see Rule 46):

When? The time limit for filing such amendments is normally 2 months from the date of transmittal of the International Search Report; however, for more details, see the notes on the accompanying sheet.

Where? Directly to the International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland
Fascimile No.: (41-22) 740.14.35

For more detailed instructions, see the notes on the accompanying sheet.

2. [ ] The applicant is hereby notified that no International Search Report will be established and that the declaration under Article 17(2)(a) to that effect is transmitted herewith.

3. [ ] **With regard to the protest** against payment of (an) additional fee(s) under Rule 40.2, the applicant is notified that:

[ ] the protest together with the decision thereon has been transmitted to the International Bureau together with the applicant's request to forward the texts of both the protest and the decision thereon to the designated Offices.

[ ] no decision has been made yet on the protest; the applicant will be notified as soon as a decision is made.

4. **Further action(s):** The applicant is reminded of the following:

Shortly after **18 months** from the priority date, the international application will be published by the International Bureau. If the applicant wishes to avoid or postpone publication, a notice of withdrawal of the international application, or of the priority claim, must reach the International Bureau as provided in Rules 90*bis*.1 and 90*bis*.3, respectively, before the completion of the technical preparations for international publication.

Within **19 months** from the priority date, a demand for international preliminary examination must be filed if the applicant wishes to postpone the entry into the national phase until 30 months from the priority date (in some Offices even later).

Within **20 months** from the priority date, the applicant must perform the prescribed acts for entry into the national phase before all designated Offices which have not been elected in the demand or in a later election within 19 months from the priority date or could not be elected because they are not bound by Chapter II.

Name and mailing address of the International Searching Authority European Patent Office, P.B. 5818 Patentlaan 2 NL-2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Authorized officer Mireille Claudepierre

Form PCT/ISA/220 (July 1998)

NOTES TO FORM PCT/ISA/220

These Notes are intended to give the basic instructions concerning the filing of amendments under article 19. The Notes are based on the requirements of the Patent Cooperation Treaty, the Regulations and the Administrative Instructions under that Treaty. In case of discrepancy between these Notes and those requirements, the latter are applicable. For more detailed information, see also the PCT Applicant's Guide, a publication of WIPO.

In these Notes, "Article", "Rule", and "Section" refer to the provisions of the PCT, the PCT Regulations and the PCT Administrative Instructions respectively.

INSTRUCTIONS CONCERNING AMENDMENTS UNDER ARTICLE 19

The applicant has, after having received the international search report, one opportunity to amend the claims of the international application. It should however be emphasized that, since all parts of the international application (claims, description and drawings) may be amended during the international preliminary examination procedure, there is usually no need to file amendments of the claims under Article 19 except where, e.g. the applicant wants the latter to be published for the purposes of provisional protection or has another reason for amending the claims before international pbulication. Furthermore, it should be emphasized that provisional protection is available in some States only.

What parts of the international application may be amended?

Under Article 19, only the claims may be amended.

During the international phase, the claims may also be amended (or further amended) under Article 34 before the International Preliminary Examining Authority. The description and drawings may only be amended under Article 34 before the International Examining Authority.

Upon entry into the national phase, all parts of the international application may be amended under Article 28 or, where applicable, Article 41.

When?

Within 2 months from the date of transmittal of the international search report or 16 months from the priority date, whichever time limit expires later. It should be noted, however, that the amendments will be considered as having been received on time if they are received by the International Bureau after the expiration of the applicable time limit but before the completion of the technical preparations for international publication (Rule 46.1).

Where not to file the amendments?

The amendments may only be filed with the International Bureau and not with the receiving Office or the International Searching Authority (Rule 46.2).

Where a demand for international preliminary examination has been/is filed, see below.

How?

Either by cancelling one or more entire claims, by adding one or more new claims or by amending the text of one or more of the claims as filed.

A replacement sheet must be submitted for each sheet of the claims which, on account of an amendment or amendments, differs from the sheet originally filed.

All the claims appearing on a replacement sheet must be numbered in Arabic numerals. Where a claim is cancelled, no renumbering of the other claims is required. In all cases where claims are renumbered, they must be renumbered consecutively (Administrative Instructions, Section 205(b)).

The amendments must be made in the language in which the international application is to be published.

What documents must/may accompany the amendments?

Letter (Section 205(b)):

The amendments must be submitted with a letter.

The letter will not be published with the international application and the amended claims. It should not be confused with the "Statement under Article 19(1)" (see below, under "Statement under Article 19(1)").

The letter must be in English or French, at the choice of the applicant. However, if the language of the international application is English, the letter must be in English; if the language of the international application is French, the letter must be in French.

The letter must indicate the differences between the claims as filed and the claims as amended. It must, in particular, indicate, in connection with each claim appearing in the international application (it being understood that identical indications concerning several claims may be grouped), whether

(i) the claim is unchanged;

(ii) the claim is cancelled;

(iii) the claim is new;

(iv) the claim replaces one or more claims as filed;

(v) the claim is the result of the division of a claim as filed.

The following examples illustrate the manner in which amendments must be explained in the accompanying letter:

1. [Where originally there were 48 claims and after amendment of some claims there are 51]:
"Claims 1 to 29, 31, 32, 34, 35, 37 to 48 replaced by amended claims bearing the same numbers; claims 30, 33 and 36 unchanged; new claims 49 to 51 added."

2. [Where originally there were 15 claims and after amendment of all claims there are 11]:
"Claims 1 to 15 replaced by amended claims 1 to 11."

3. [Where originally there were 14 claims and the amendments consist in cancelling some claims and in adding new claims]:
"Claims 1 to 6 and 14 unchanged; claims 7 to 13 cancelled; new claims 15, 16 and 17 added." or
"Claims 7 to 13 cancelled; new claims 15, 16 and 17 added; all other claims unchanged."

4. [Where various kinds of amendments are made]:
"Claims 1-10 unchanged; claims 11 to 13, 18 and 19 cancelled; claims 14, 15 and 16 replaced by amended claim 14; claim 17 subdivided into amended claims 15, 16 and 17; new claims 20 and 21 added."

"Statement under article 19(1)" (Rule 46.4)

The amendments may be accompanied by a statement explaining the amendments and indicating any impact that such amendments might have on the description and the drawings (which cannot be amended under Article 19(1)).

The statement will be published with the international application and the amended claims.

It must be in the language in which the international appplication is to be published.

It must be brief, not exceeding 500 words if in English or if translated into English.

It should not be confused with and does not replace the letter indicating the differences between the claims as filed and as amended. It must be filed on a separate sheet and must be identified as such by a heading, preferably by using the words "Statement under Article 19(1)."

It may not contain any disparaging comments on the international search report or the relevance of citations contained in that report. Reference to citations, relevant to a given claim, contained in the international search report may be made only in connection with an amendment of that claim.

Consequence if a demand for international preliminary examination has already been filed

If, at the time of filing any amendments under Article 19, a demand for international preliminary examination has already been submitted, the applicant must preferably, at the same time of filing the amendments with the International Bureau, also file a copy of such amendments with the International Preliminary Examining Authority (see Rule 62.2(a), first sentence).

Consequence with regard to translation of the international application for entry into the national phase

The applicant's attention is drawn to the fact that, where upon entry into the national phase, a translation of the claims as amended under Article 19 may have to be furnished to the designated/elected Offices, instead of, or in addition to, the translation of the claims as filed.

For further details on the requirements of each designated/elected Office, see Volume II of the PCT Applicant's Guide.

PATENT COOPERATION TREAT

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference	FOR FURTHER ACTION see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.	
08-887547WO		
International application No.	International filing date *(day/month/year)*	(Earliest) Priority Date *(day/month/year)*
PCT/CA 00/ 00642	02/06/2000	02/06/1999

Applicant

PLANT RESEARCH INTERNATIONAL et al.

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 4 sheets.

[X] It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**

   a. With regard to the **language**, the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

   [ ] the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

   b. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international search was carried out on the basis of the sequence listing :

   [X] contained in the international application in written form.

   [ ] filed together with the international application in computer readable form.

   [ ] furnished subsequently to this Authority in written form.

   [X] furnished subsequently to this Authority in computer readble form.

   [X] the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

   [X] the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

2. [ ] **Certain claims were found unsearchable** (See Box I).

3. [ ] **Unity of invention is lacking** (see Box II).

4. With regard to the **title,**

   [ ] the text is approved as submitted by the applicant.

   [X] the text has been established by this Authority to read as follows:

   USE OF THE BNM3 TRANSCRIPTIONAL ACTIVATOR TO CONTROL PLANT EMBRYOGENESIS AND REGENERATION PROCESSES

5. With regard to the **abstract,**

   [X] the text is approved as submitted by the applicant.

   [ ] the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is Figure No. 1

   [X] as suggested by the applicant.

   [ ] because the applicant failed to suggest a figure.

   [ ] because this figure better characterizes the invention.

   [ ] None of the figures.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/CA 00/00642

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12N15/29 C12N15/82 C12N5/10 C07K14/415 A01H5/00
A01H5/10

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C12N C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

CHEM ABS Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	NAKAMURA Y.: "AC AB025629" EMBL DATABASE, 9 April 1999 (1999-04-09), XP002120887 Heidelberg the whole document ---	1
X	ELLIOTT R. ET AL.: "AINTEGUMENTA, an APETALA2-like gene of arabidopsis with pleiotropic roles in ovule development and floran organ growth" THE PLANT CELL, vol. 8, 1996, pages 155-168, XP002120889 cited in the application * see esp. p.161;165 * ---	54
	-/--	

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
10 October 2000	03/11/2000

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Kania, T

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DATABASE EBI 'Online! AC AL008970, 18 November 1997 (1997-11-18) HAMLIN N. ET AL.: "Plasmodium falciparum MAL3P4" XP002149645 abstract	63
A	RIEGER M. ET AL.: "AC AL049862" EMBL DATABASE, 13 May 1999 (1999-05-13), XP002120888 Heidelberg the whole document	28,29
A	WO 97 43427 A (CIBA GEIGY AG ;VRIES SAPE CORNELIS DE (NL); SCHMIDT EDUARD DANIEL) 20 November 1997 (1997-11-20) cited in the application the whole document	1-62
A	WO 98 37184 A (UNIV CALIFORNIA) 27 August 1998 (1998-08-27) * see esp. example 2 *	1-62
A	WO 98 07842 A (JOFUKU K DIANE ;OKAMURO JACK K (US); UNIV CALIFORNIA (US)) 26 February 1998 (1998-02-26) the whole document	1-62
A	OGAS J. ET AL.: "Cellular differentiation regulated by gibberellin in the Arabidopsis thaliana pickle mutant" SCIENCE, vol. 277, July 1997 (1997-07), pages 91-94, XP002120890 cited in the application the whole document	1-62
A	BOUTILIER, K. A. ET AL: "Expression of the BnmNAP subfamily of napin genes coincides with the induction of Brassica microspore embryogenesis" PLANT MOLECULAR BIOLOGY, (1994) VOL. 26, NO. 6, PP. 1711-1723. 48 REF. ISSN: 0167-4412, XP002120891 cited in the application the whole document	1-62

-/--

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	CUSTERS, JAN B.M. ET AL: "Regulation of the inductive phase of microspore embryogenesis in Brassica napus" ACTA HORTIC. (1996), 407(INTERNATIONAL SYMPOSIUM ON BRASSICAS, NINTH CRUCIFER GENETICS WORKSHOP, 1994), 209-217, XP002120892 * see the whole document; esp. table 3 clone 13 * ---	1-62
A	CHAUDHURY A. ET AL.: "Ovule and embryo development, apomixis and fertilization" CURRENT OPINION IN PLANT BIOLOGY, 'Online! vol. 1, 1998, pages 26-31, XP002120893 Retrieved from the Internet: <URL:http://biomednet.com/elecref/1369526600100026> 'retrieved on 1999-10-27! * see esp. p.29 * -----	1-62

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/CA 00/00642

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9743427 A	20-11-1997	AU 713130 B	25-11-1999
		AU 2953997 A	05-12-1997
		BR 9709098 A	03-08-1999
		CA 2254839 A	20-11-1997
		CN 1218510 A	02-06-1999
		EP 0915984 A	19-05-1999
		HU 9901477 A	28-09-1999
		PL 329872 A	12-04-1999
WO 9837184 A	27-08-1998	AU 6328398 A	09-09-1998
		EP 0977836 A	09-02-2000
WO 9807842 A	26-02-1998	US 5994622 A	30-11-1999
		AU 3986797 A	06-03-1998
		EP 0964920 A	22-12-1999
		PL 331771 A	02-08-1999
		US 6093874 A	25-07-2000

PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF THE RECORDING OF A CHANGE

(PCT Rule 92bis.1 and Administrative Instructions, Section 422)

To:

SECHLEY, Konrad, A.
Gowling Lafleur Henderson LLP
Suite 2600
160 Elgin Street
Ottawa, Ontario K1P 1C3
CANADA

Date of mailing (day/month/year)
11 October 2000 (11.10.00)

Applicant's or agent's file reference
08-887547WO

IMPORTANT NOTIFICATION

International application No.
PCT/CA00/00642

International filing date (day/month/year)
02 June 2000 (02.06.00)

1. The following indications appeared on record concerning:

[X] the applicant [X] the inventor [ ] the agent [ ] the common representative

Name and Address
VAN LOOKEREN CAMPAGNE, Michiel
Lievestraat 25
B-9920 Lovendegem
Belgium

State of Nationality: BE
State of Residence: NL
Telephone No.:
Facsimile No.:
Teleprinter No.:

2. The International Bureau hereby notifies the applicant that the following change has been recorded concerning:

[ ] the person [ ] the name [X] the address [ ] the nationality [ ] the residence

Name and Address
VAN LOOKEREN CAMPAGNE, Michiel
Lievestraat 31
B-9920 Lovendegem
Belgium

State of Nationality: BE
State of Residence: NL
Telephone No.:
Facsimile No.:
Teleprinter No.:

3. Further observations, if necessary:

4. A copy of this notification has been sent to:

[X] the receiving Office
[X] the International Searching Authority
[ ] the International Preliminary Examining Authority
[ ] the designated Offices concerned
[ ] the elected Offices concerned
[ ] other:

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland

Facsimile No.: (41-22) 740.14.35

Authorized officer
Aino Metcalfe

Telephone No.: (41-22) 338.83.38

PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

To:

SECHLEY, Konrad, A.
Gowling Lafleur Henderson LLP
Suite 2600
160 Elgin Street
Ottawa, Ontario K1P 1C3
CANADA

INFORMATION CONCERNING ELECTED OFFICES NOTIFIED OF THEIR ELECTION

(PCT Rule 61.3)

Date of mailing (day/month/year) 09 February 2001 (09.02.01)	
Applicant's or agent's file reference 08-887547WO	**IMPORTANT INFORMATION**

International application No.	**International filing date** (day/month/year)	**Priority date** (day/month/year)
PCT/CA00/00642	02 June 2000 (02.06.00)	02 June 1999 (02.06.99)

Applicant
PLANT RESEARCH INTERNATIONAL et al

1. The applicant is hereby informed that the International Bureau has, according to Article 31(7), notified each of the following Offices of its election:

   AP :GH,GM,KE,LS,MW,MZ,SD,SL,SZ,TZ,UG,ZW
   EP :AT,BE,CH,CY,DE,DK,ES,FI,FR,GB,GR,IE,IT,LU,MC,NL,PT,SE
   National :AU,BG,CA,CN,CZ,DE,IL,JP,KP,KR,MN,NO,NZ,PL,RO,RU,SE,SK,US

2. The following Offices have waived the requirement for the notification of their election; the notification will be sent to them by the International Bureau only upon their request:

   EA :AM,AZ,BY,KG,KZ,MD,RU,TJ,TM
   OA :BF,BJ,CF,CG,CI,CM,GA,GN,GW,ML,MR,NE,SN,TD,TG
   National :AE,AG,AL,AM,AT,AZ,BA,BB,BR,BY,CH,CR,CU,DK,DM,DZ,EE,ES,FI,GB,GD,
   GE,GH,GM,HR,HU,ID,IN,IS,KE,KG,KZ,LC,LK,LR,LS,LT,LU,LV,MA,MD,MG,MK,MW,MX,
   PT,SD,SG,SI,SL,TJ,TM,TR,TT,TZ,UA,UG,UZ,VN,YU,ZA,ZW

3. The applicant is reminded that he must enter the "national phase" **before the expiration of 30 months from the priority date** before each of the Offices listed above. This must be done by paying the national fee(s) and furnishing , if prescribed, a translation of the international application (Article 39(1)(a)), as well as, where applicable, by furnishing a translation of any annexes of the international preliminary examination report (Article 36(3)(b) and Rule 74.1).

   Some offices have fixed time limits expiring later than the above-mentioned time limit. For detailed information about the applicable time limits and the acts to be performed upon entry into the national phase before a particular Office, see Volume II of the PCT Applicant's Guide.

   The entry into the European regional phase is postponed **until 31 months from the priority date** for all States designated for the purposes of obtaining a European patent.

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland

Facsimile No. (41-22) 740.14.35

Authorized officer:
A. Karkachi

Telephone No. (41-22) 338.83.38

PCT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION CONCERNING SUBMISSION OR TRANSMITTAL OF PRIORITY DOCUMENT

(PCT Administrative Instructions, Section 411)

To:

SECHLEY, Konrad, A.
Gowling Lafleur Henderson LLP
Suite 2600
160 Elgin Street
Ottawa, Ontario K1P 1C3
CANADA

BY: _____

Date of mailing (day/month/year) 11 October 2000 (11.10.00)	
Applicant's or agent's file reference 08-887547WO	**IMPORTANT NOTIFICATION**
International application No. PCT/CA00/00642	International filing date (day/month/year) 02 June 2000 (02.06.00)
International publication date (day/month/year) Not yet published	Priority date (day/month/year) 02 June 1999 (02.06.99)
Applicant PLANT RESEARCH INTERNATIONAL et al	

1. The applicant is hereby notified of the date of receipt (except where the letters "NR" appear in the right-hand column) by the International Bureau of the priority document(s) relating to the earlier application(s) indicated below. Unless otherwise indicated by an asterisk appearing next to a date of receipt, or by the letters "NR", in the right-hand column, the priority document concerned was submitted or transmitted to the International Bureau in compliance with Rule 17.1(a) or (b).

2. This updates and replaces any previously issued notification concerning submission or transmittal of priority documents.

3. An **asterisk**(*) appearing next to a date of receipt, in the right-hand column, denotes a priority document submitted or transmitted to the International Bureau but not in compliance with Rule 17.1(a) or (b). In such a case, **the attention of the applicant is directed** to Rule 17.1(c) which provides that no designated Office may disregard the priority claim concerned before giving the applicant an opportunity, upon entry into the national phase, to furnish the priority document within a time limit which is reasonable under the circumstances.

4. The **letters "NR"** appearing in the right-hand column denote a priority document which was not received by the International Bureau or which the applicant did not request the receiving Office to prepare and transmit to the International Bureau, as provided by Rule 17.1(a) or (b), respectively. In such a case, **the attention of the applicant is directed** to Rule 17.1(c) which provides that no designated Office may disregard the priority claim concerned before giving the applicant an opportunity, upon entry into the national phase, to furnish the priority document within a time limit which is reasonable under the circumstances.

Priority date	Priority application No.	Country or regional Office or PCT receiving Office	Date of receipt of priority document
02 June 1999 (02.06.99)	99201745.9	EP	13 Sept 2000 (13.09.00)

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland

Facsimile No. (41-22) 740.14.35

Authorized officer

Aino Metcalfe

Telephone No. (41-22) 338.83.38

PATENT COOPERATION TREATY

From the:
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

To:

SECHLEY, Konrad A. et al.
Gowling Lafleur Henderson LLP
Suite 2600
160 Elgin Street
Ottawa, Ontario K1P 1C3
CANADA

WRITTEN OPINION

(PCT Rule 66)

Date of mailing *(day/month/year)*	15.05.2001
Applicant's or agent's file reference: 08-887547WO	**REPLY DUE** **within 3 month(s)** from the above date of mailing

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/CA00/00642	02/06/2000	02/06/1999

International Patent Classification (IPC) or both national classification and IPC

C12N15/29

Applicant

PLANT RESEARCH INTERNATIONAL et al.

1. This written opinion is the **first** drawn up by this International Preliminary Examining Authority.

2. This opinion contains indications relating to the following items:

I	☒	Basis of the opinion
II	☐	Priority
III	☐	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
IV	☐	Lack of unity of invention
V	☒	Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
VI	☐	Certain document cited
VII	☐	Certain defects in the international application
VIII	☒	Certain observations on the international application

3. The applicant is hereby **invited to reply** to this opinion.

When? See the time limit indicated above. The applicant may, before the expiration of that time limit, request this Authority to grant an extension, see Rule 66.2(d).

How? By submitting a written reply, accompanied, where appropriate, by amendments, according to Rule 66.3. For the form and the language of the amendments, see Rules 66.8 and 66.9.

Also: For an additional opportunity to submit amendments, see Rule 66.4.
For the examiner's obligation to consider amendments and/or arguments, see Rule 66.4 bis.
For an informal communication with the examiner, see Rule 66.6.

If no reply is filed, the international preliminary examination report will be established on the basis of this opinion.

4. The final date by which the international preliminary examination report must be established according to Rule 69.2 is: 02/10/2001.

Name and mailing address of the international preliminary examining authority:
European Patent Office
D-80298 Munich
Tel. +49 89 2399 - 0 Tx: 523656 epmu d
Fax: +49 89 2399 - 4465

Authorized officer / Examiner
Young, C

Formalities officer (incl. extension of time limits)
Emslander, S
Telephone No. +49 89 2399 8718

I. Basis of the opinion

1. With regard to the **elements** of the international application (Replacement *sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this opinion as "originally filed")*

Description, pages:

1-69 as originally filed

Claims, No.:

1-74 as originally filed

Drawings, sheets:

1-11 as originally filed

Sequence listing part of the description, pages:

1-15, filed with the letter of 27.9.2000

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language: , which is:

- ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
- ☐ the language of publication of the international application (under Rule 48.3(b)).
- ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rul: 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

- ☐ contained in the international application in written form.
- ☐ filed together with the international application in computer readable form.
- ☒ furnished subsequently to this Authority in written form.
- ☒ furnished subsequently to this Authority in computer readable form.
- ☒ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
- ☒ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

- ☐ the description, pages:
- ☐ the claims, Nos.:
- ☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

   (Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

V. Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Claims	1,2,28,29,54
Inventive step (IS)	Claims	
Industrial applicability (IA)	Claims	

2. Citations and explanations
   see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:
see separate sheet

The present application concerns sequences from Brassica and Arapidopsis which when expressed in plant cells renders cells embryogenic. The genes possesses the highly conserved plant homeotic domain repeats known as the AP2 repeat.

Reference is made to the following documents:

D1: NAKAMURA Y.: 'AC AB025629' EMBL DATABASE, 9 April 1999 (1999-04-09), XP002120887 Heidelberg

D2: ELLIOTT R. ET AL.: 'AINTEGUMENTA, an APETALA2-like gene of arabidopsis with pleiotropic roles in ovule development and floral organ growth' THE PLANT CELL, vol. 8, 1996, pages 155-168, XP002120889 cited in the application

Re Item V

Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

Novelty, Article 33 (2) PCT.

D1 discloses an isolated DNA molecule capable of hybridising to the DNA sequence of SEQ ID's 1, 3 and 5 under stringent hybridisation conditions. Furthermore D1 possesses at least 27 contiguous nucleotides which are identical to a nucleotide sequence selected from the group consisting of SEQ ID 1, 3 and 5. Therefore claims 1, 2, 28 and 29 are not novel over D1. Consequently these claims do not meet with the requirements for novelty as set out under article 33 (2) PCT.
D2 discloses an isolated DNA molecule comprising a sequence encoding a protein bearing two AP2 DNA binding domains which is involved in very early organ development and primordial growth, see page 165 of D2. As such it is considered that this protein increases the plant's regenerative capacity. Correspondingly D2 destroys the novelty of claim 54 and as such this claim fails to meet the requirements of Article 33 (2) PCT.

Inventive step, Article 33 (3) PCT.

The closest prior art is considered to be D2. D2 discloses the cloning of the ANT gene.

The authors characterise the gene as being involved in ovule development and floral growth. Moreover the gene bears two tandemly repeated AP2 domains and most certainly functions as a transcription factor promoting primordial regeneration.
The present application concerns a gene which encodes a protein containing two AP2 domains which is capable of rendering a plant cell embryogenic. This property is not described for ANT or homologues disclosed in D2.
The objective problem is defined as;
To provide a gene which when expressed renders plant cells embryogenic.

No indication exists in the cited prior art that such a gene exists. Thus the cloning of this gene is considered to contain an inventive step. As such claims which were not indicated above as lacking novelty are considered as meeting the requirements of Article 33 (3) PCT.

Re Item VIII

Claims 3 and 4 are directed to an isolated DNA with a percentage homology with a nucleotide sequence. It is unclear how homology is defined which is understood by the skilled person as a synonym for similarity. The applicant is thus kindly requested to express this term as percentage identity

In order to expedite proceedings and in view of the short time available under PCT procedures, applicants should ensure that they deal with all issues raised in this written opinion in their reply.

PATENT COOPERATION TREATY

14

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
08-887547WO		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/CA00/00642	02/06/2000	02/06/1999
International Patent Classification (IPC) or national classification and IPC C12N15/29		
Applicant PLANT RESEARCH INTERNATIONAL *et al.*		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 5 sheets, including this cover sheet.

   ☐ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☒ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
13/12/2000	04.09.2001
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized officer Young, C Telephone No. +49 89 2399 7677

Form PCT/IPEA/409 (cover sheet) (January 1994)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/CA00/00642

I. Basis of the report

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:
Description, pages:

1-69 as originally filed

Claims, No.:

1-74 as originally filed

Drawings, sheets:

1-11 as originally filed

Sequence listing part of the description, pages:

1-15, filed with the letter of 27.9.2000

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language: , which is:

- ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
- ☐ the language of publication of the international application (under Rule 48.3(b)).
- ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

- ☐ contained in the international application in written form.
- ☐ filed together with the international application in computer readable form.
- ☒ furnished subsequently to this Authority in written form.
- ☒ furnished subsequently to this Authority in computer readable form.
- ☒ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
- ☒ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

- ☐ the description, pages:
- ☐ the claims, Nos.:
- ☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

   (Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. *Additional observations, if necessary:*

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-53,55-74
	No:	Claims	54
Inventive step (IS)	Yes:	Claims	4-27,30-53,55-74
	No:	Claims	1-3,28,29
Industrial applicability (IA)	Yes:	Claims	1-74
	No:	Claims	

2. Citations and explanations
   see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:
see separate sheet

INTERNATIONAL PRELIMINARY EXAMINATION REPORT - SEPARATE SHEET

International application No. PCT/CA00/00642

Reference is made to the following documents:

D1:NAKAMURA Y.: 'AC AB025629' EMBL DATABASE, 9 April 1999 (1999-04-09), Heidelberg.

D2:ELLIOTT R. ET AL.: 'AINTEGUMENTA, an APETALA2-like gene of arabidopsis with pleiotropic roles in ovule development and floral organ growth' THE PLANT CELL, vol. 8, 1996, pages 155-168, cited in the application

Re Item V

Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

Novelty, Article 33 (2) PCT.

D1 discloses an isolated DNA molecule capable of hybridising to the DNA sequence of SEQ ID's 1, 3 and 5 under stringent hybridisation conditions. Furthermore D1 possesses at least 27 contiguous nucleotides which are identical to a nucleotide sequence selected from the group consisting of SEQ ID 1, 3 and 5. However, the disclaimer excluding an AP2 domain repeat 1-linker-AP2 domain repeat 2 region renders claim 1, 2, 28 and 29 novel as the said sequence from D1 hybridises within the AP2 region of the aforementioned SEQ IDs. Consequently these claims and dependant claims thereof meet with the requirements for novelty as set out under Article 33 (2) PCT. Claims 4 and dependant claims thereof relate to a DNA sequence encoding a protein which is capable of rendering a plant embryogenic. These claims do not disclaim the AP2 domain. The functional restriction does however confer novelty and as such the requirement of Article 33 (2) PCT is met.

D2 discloses an isolated DNA molecule comprising a sequence encoding a protein bearing two AP2 DNA binding domains which is involved in very early organ development and primordial growth, see page 165 of D2. As such it is considered that this protein increases the plant's regenerative capacity. Correspondingly D2 destroys the novelty of claim 54 and as such this claim fails to meet the requirements of Article 33 (2) PCT.

Inventive step, Article 33 (3) PCT.

The closest prior art is considered to be D2. D2 discloses the cloning of the ANT gene. The authors characterise the gene as being involved in ovule development and floral growth. Moreover the gene bears two tandemly repeated AP2 domains and most certainly functions as a transcription factor promoting primordial regeneration.
The present application concerns a gene which encodes a protein containing two AP2 domains which is capable of rendering a plant cell embryogenic. This property is not described for ANT or homologues disclosed in D2.
The objective problem is defined as;
To provide a gene which when expressed renders plant cells embryogenic.

Claims 1, 2, 3, 28 and 29 are directed to DNA sequences whereby the AP2 encoding domain is disclaimed. It is considered that as it is known in the art that this conserved domain is present in many developmentally important genes it would seem highly improbable that the claimed sequences would indeed solve the objective problem. Moreover, the Applicant does not provide any experimental evidence which would indicate anything to the contrary. Thus, claims 1, 2, 3, 28 and 29 are construed as not having an inventive step and therefore are seen not to comply with Article 33 (3) PCT.

However, claim 4 and dependant claims thereof are directed to the DNA sequence bearing the AP domains. The alleged properties of this gene would indeed solve the technical problem. No indication exists in the cited prior art that such a gene exists. Thus the cloning of this gene is considered to contain an inventive step. As such said claims are considered as meeting the requirements of Article 33 (3) PCT.

Re Item VIII

Claims 3 and 4 are directed to an isolated DNA with a percentage homology to a nucleotide sequence. As the skilled person may consider this to be a synonym for similarity the exact meaning of homology is unclear from the claims alone. Thus, the scope of protection sought is not sufficiently clear to meet the requirements of Article 6 PCT.

by fax and post

From the
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

PCT

To:

SECHLEY, Konrad A. et al.
Gowling Lafleur Henderson LLP
Suite 2600
160 Elgin Street
Ottawa, Ontario K1P 1C3
CANADA

Fax: 613 563 9869

NOTIFICATION OF TRANSMITTAL OF THE INTERNATIONAL PRELIMINARY EXAMINATION REPORT
(PCT Rule 71.1)

Date of mailing *(day/month/year)*	04.09.2001
Applicant's or agent's file reference 08-887547WO	IMPORTANT NOTIFICATION

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/CA00/00642	02/06/2000	02/06/1999

Applicant
PLANT RESEARCH INTERNATIONAL et al.

1. The applicant is hereby notified that this International Preliminary Examining Authority transmits herewith the international preliminary examination report and its annexes, if any, established on the international application.

2. A copy of the report and its annexes, if any, is being transmitted to the International Bureau for communication to all the elected Offices.

3. Where required by any of the elected Offices, the International Bureau will prepare an English translation of the report (but not of any annexes) and will transmit such translation to those Offices.

4. **REMINDER**

The applicant must enter the national phase before each elected Office by performing certain acts (filing translations and paying national fees) within 30 months from the priority date (or later in some Offices) (Article 39(1)) (see also the reminder sent by the International Bureau with Form PCT/IB/301).

Where a translation of the international application must be furnished to an elected Office, that translation must contain a translation of any annexes to the international preliminary examination report. It is the applicant's responsibility to prepare and furnish such translation directly to each elected Office concerned.

For further details on the applicable time limits and requirements of the elected Offices, see Volume II of the PCT Applicant's Guide.

Name and mailing address of the IPEA/	Authorized officer
European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Guerin, A Tel.+49 89 2399-8061

Form PCT/IPEA/416 (July 1992)

[redacted]NT COOPERATION TREA[redacted]

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
08-887547WO		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/CA00/00642	02/06/2000	02/06/1999
International Patent Classification (IPC) or national classification and IPC C12N15/29		
Applicant PLANT RESEARCH INTERNATIONAL et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 6 sheets, including this cover sheet.

   ☐ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☒ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
13/12/2000	04.09.2001
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized officer Young, C Telephone No. +49 89 2399 7877

Form PCT/IPEA/409 (cover sheet) (January 1994)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/CA00/00642

I. Basis of th report

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:
Description, pages:

1-69 as originally filed

Claims, No.:

1-74 as originally filed

Drawings, sheets:

1-11 as originally filed

Sequence listing part of the description, pages:

1-15, filed with the letter of 27.9.2000

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language: , which is:

- ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
- ☐ the language of publication of the international application (under Rule 48.3(b)).
- ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

- ☐ contained in the international application in written form.
- ☐ filed together with the international application in computer readable form.
- ☒ furnished subsequently to this Authority in written form.
- ☒ furnished subsequently to this Authority in computer readable form.
- ☒ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
- ☒ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/CA00/00642

- ☐ the description, pages:
- ☐ the claims, Nos.:
- ☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

   (Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-53,55-74
	No:	Claims	54
Inventive step (IS)	Yes:	Claims	4-27,30-53,55-74
	No:	Claims	1-3,28,29
Industrial applicability (IA)	Yes:	Claims	1-74
	No:	Claims	

2. Citations and explanations
   see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:
see separate sheet

INTERNATIONAL PRELIMINARY EXAMINATION REPORT - SEPARATE SHEET

International application No. PCT/CA00/00642

Reference is made to the following documents:

D1:NAKAMURA Y.: 'AC AB025629' EMBL DATABASE, 9 April 1999 (1999-04-09), Heidelberg.

D2:ELLIOTT R. ET AL.: 'AINTEGUMENTA, an APETALA2-like gene of arabidopsis with pleiotropic roles in ovule development and floral organ growth' THE PLANT CELL, vol. 8, 1996, pages 155-168, cited in the application

Re Item V

Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

Novelty, Article 33 (2) PCT.

D1 discloses an isolated DNA molecule capable of hybridising to the DNA sequence of SEQ ID's 1, 3 and 5 under stringent hybridisation conditions. Furthermore D1 possesses at least 27 contiguous nucleotides which are identical to a nucleotide sequence selected from the group consisting of SEQ ID 1, 3 and 5. However, the disclaimer excluding an AP2 domain repeat 1-linker-AP2 domain repeat 2 region renders claim 1, 2, 28 and 29 novel as the said sequence from D1 hybridises within the AP2 region of the aforementioned SEQ IDs. Consequently these claims and dependant claims thereof meet with the requirements for novelty as set out under Article 33 (2) PCT. Claims 4 and dependant claims thereof relate to a DNA sequence encoding a protein which is capable of rendering a plant embryogenic. These claims do not disclaim the AP2 domain. The functional restriction does however confer novelty and as such the requirement of Article 33 (2) PCT is met.

D2 discloses an isolated DNA molecule comprising a sequence encoding a protein bearing two AP2 DNA binding domains which is involved in very early organ development and primordial growth, see page 165 of D2. As such it is considered that this protein increases the plant's regenerative capacity. Correspondingly D2 destroys the novelty of claim 54 and as such this claim fails to meet the requirements of Article 33 (2) PCT.

INTERNATIONAL PRELIMINARY EXAMINATION REPORT - SEPARATE SHEET

International application No. PCT/CA00/00642

Inventive step, Article 33 (3) PCT.

The closest prior art is considered to be D2. D2 discloses the cloning of the ANT gene. The authors characterise the gene as being involved in ovule development and floral growth. Moreover the gene bears two tandemly repeated AP2 domains and most certainly functions as a transcription factor promoting primordial regeneration.
The present application concerns a gene which encodes a protein containing two AP2 domains which is capable of rendering a plant cell embryogenic. This property is not described for ANT or homologues disclosed in D2.
The objective problem is defined as;
To provide a gene which when expressed renders plant cells embryogenic.

Claims 1, 2, 3, 28 and 29 are directed to DNA sequences whereby the AP2 encoding domain is disclaimed. It is considered that as it is known in the art that this conserved domain is present in many developmentally important genes it would seem highly improbable that the claimed sequences would indeed solve the objective problem. Moreover, the Applicant does not provide any experimental evidence which would indicate anything to the contrary. Thus, claims 1, 2, 3, 28 and 29 are construed as not having an inventive step and therefore are seen not to comply with Article 33 (3) PCT.

However, claim 4 and dependant claims thereof are directed to the DNA sequence bearing the AP domains. The alleged properties of this gene would indeed solve the technical problem. No indication exists in the cited prior art that such a gene exists. Thus the cloning of this gene is considered to contain an inventive step. As such said claims are considered as meeting the requirements of Article 33 (3) PCT.

Re Item VIII

Claims 3 and 4 are directed to an isolated DNA with a percentage homology to a nucleotide sequence. As the skilled person may consider this to be a synonym for similarity the exact meaning of homology is unclear from the claims alone. Thus, the scope of protection sought is not sufficiently clear to meet the requirements of Article 6 PCT.

PCT

REQUEST

The undersigned requests that the present international application be processed according to the Patent Cooperation Treaty.

For receiving Office use only

International Application No. PCT / CA 00 / 00642

International Filing Date 02 JUN 2000 (02.06.00)

Name of receiving Office and "PCT International Application" RO/CA

Applicant's or agent's file reference *(if desired) (12 characters maximum)* O8-887547WO

Box No. I TITLE OF INVENTION

Use of The *BNM3* Transcriptional Activator To Control Plant Embryogenesis And Regenration Processes

Box No. II APPLICANT

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

PLANT RESEARCH INTERNATIONAL
Building No. 107
Droevendaalsesteeg 1
6708 PB Wageningen
THE NETHERLANDS

☐ This person is also inventor.

Telephone No.
(31) 317 47 70 01

Facsimile No.
(31) 317 41 80 94

Teleprinter No.

State (that is, *country*) of nationality: NL

State (that is, *country*) of residence: NL

This person is applicant for the purposes of:
☒ all designated states
☒ all designated States except the United States of America
☐ the United States of America only
☐ the States indicated in the Supplemental Box

Box No. III FURTHER APPLICANT(S) AND/OR (FURTHER) INVENTOR(S)

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

Her Majesty The Queen In Right Of Canada, As Represented By The Minister Of Agriculture And Agri-Food Canada
Eastern Cereal & Oilseed Research Centre
K.W. Neatby Building
Ottawa, Ontario
CANADA, K1A 0C6

This person is:
☒ applicant only
☐ applicant and inventor
☐ inventor only *(If this check-box is marked, do not fill in below.)*

State (that is, *country*) of nationality: CA

State (*that is, country*) of residence: CA

This person is applicant for the purposes of:
☒ all designated states
☒ all designated States except the United States of America
☐ the United States of America only
☐ the States indicated in the Supplemental Box

☒ Further applicants and/or (further) inventors are indicated on a continuation sheet.

Box No. IV AGENT OR COMMON REPRESENTATIVE; OR ADDRESS FOR CORRESPONDENCE

The person identified below is hereby/has been appointed to act on behalf of the applicant(s) before the competent International Authorities as: ☒ agent ☐ common representative

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

SECHLEY, Konrad A.; ERRATT, Judy A.; DUDLEY, Bruce; MORGAN, Bruce E.; STRAZNICKY, Ivan; D'IORIO, Hélène; O'NEILL, Gary; WADA, Ikuko; HARRIS, John D.
Gowling, Strathy & Henderson
Suite 2600, 160 Elgin Street
Ottawa, Ontario
Canada K1P 1C3

Telephone No.
(613) 233-1781

Facsimile No.
(613) 563-9869

Teleprinter No.

☐ **Address for correspondence:** Mark this check-box where no agent or common representative is/has been appointed and the space above is used instead to indicate a special address to which correspondence should be sent.

RO/CA

DELETED RO/CA

Continuation of Box No. III FURTHER APPLICANTS AND/OR (FURTHER) INVENTORS	
If none of the following sub-boxes is used, this sheet is not to be included in the request.	
Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)* **BOUTILER, Kim** Groen van Prinstererstraat 87 6702 CP Wageningen The Netherlands	This person is: ☐ applicant only ☒ applicant and inventor ☐ inventor only (*If this check-box is marked, do not fill in below.*)
State (that is, *country*) of nationality: CA	**State (that is, *country*) of residence:** NL
This person is applicant for the purposes of: ☐ all designated states ☐ all designated States except the United States of America ☒ the United States of America only ☐ the States indicated in the Supplemental Box	
Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal codeand name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated bebw.)* **OUELLET, Thérèse** 115 Kennevale Drive Nepean, Ontario CANADA, K2J 3X7	This person is: ☐ applicant only ☒ applicant and inventor ☐ inventor only (*If this check-box is marked, do not fill in below.*)
State (that is, *country*) of nationality: CA	**State (that is, *country*) of residence:** CA
This person is applicant for the purposes of: ☐ all designated states ☐ all designated States except the United States of America ☒ the United States of America only ☐ the States indicated in the Supplemental Box	
Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal codeand name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated bebw.)* **CUSTERS, Jan** Gen. Foulkesweg 25 6703 BL Wageningen The Neatherlands	This person is: ☐ applicant only ☒ applicant and inventor ☐ inventor only (*If this check-box is marked, do not fill in below.*)
State (*that is, country*) of nationality: NL	**State (*that is, country*) of residence:** NL
This person is applicant for the purposes of: ☐ all designated states ☐ all designated States except the United States of America ☒ the United States of America only ☐ the States indicated in the Supplemental Box	
Name and address: *(Family name followed by given name; for a legal entity full official designation. The address must includepostal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)* **HATTORI, Jiro** 763 Halstead Street Ottawa, Ontario CANADA, K1G 1M5	This person is: ☐ applicant only ☒ applicant and inventor ☐ inventor only (*If this check-box is marked, do not fill in below.*)
State (*that is, country*) of nationality: JP	**State (*that is, country*) of residence:** CA
This person is applicant for the purposes of: ☐ all designated states ☐ all designated States except the United States of America ☒ the United States of America only ☐ the States indicated in the Supplemental Box	
☒ Further applicants and/or (further) inventors are indicated on another continuation sheet.	

Continuation of Box No. III FURTHER APPLICANTS AND/OR (FURTHER) INVENTORS	
If none of the following sub-boxes is used, this sheet is not to be included in the request.	
Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)* **MIKI, Brian** 1876 Dorset Drive Ottawa, Ontario CANADA, K1H 5V1	This person is: ☐ applicant only ☒ applicant and inventor ☐ inventor only (*If this check-box is marked, do not fill in below.*)
State (that is, *country*) of nationality: CA	**State (that is, *country*) of residence:** CA
This person is applicant for the purposes of: ☐ all designated states ☐ all designated States except the United States of America ☒ the United States of America only ☐ the States indicated in the Supplemental Box	
Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal codeand name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated bebw.)* **VAN LOOKEREN CAMPAGNE, Michiel** Lievestraat 25 B-9920 Lovendegem Belgium THE NETHERLANDS	This person is: ☐ applicant only ☒ applicant and inventor ☐ inventor only (*If this check-box is marked, do not fill in below.*)
State (that is, *country*) of nationality: NL	**State (that is, *country*) of residence:** BE
This person is applicant for the purposes of: ☐ all designated states ☐ all designated States except the United States of America ☒ the United States of America only ☐ the States indicated in the Supplemental Box	
Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal codeand name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated bebw.)*	This person is: ☐ applicant only ☐ applicant and inventor ☐ inventor only (*If this check-box is marked, do not fill in below.*)
State (*that is, country*) of nationality:	**State (*that is, country*) of residence:**
This person is applicant for the purposes of: ☐ all designated states ☐ all designated States except the United States of America ☐ the United States of America only ☐ the States indicated in the Supplemental Box	
Name and address: *(Family name followed by given name; for a legal entity full official designation. The address must includepostal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*	This person is: ☐ applicant only ☐ applicant and inventor ☐ inventor only (*If this check-box is marked, do not fill in below.*)
State (*that is, country*) of nationality:	**State (*that is, country*) of residence:**
This person is applicant for the purposes of: ☐ all designated states ☐ all designated States except the United States of America ☐ the United States of America only ☐ the States indicated in the Supplemental Box	
☐ Further applicants and/or (further) inventors are indicated on another continuation sheet.	

Box No. V DESIGNATION OF STATES

The following designations are hereby made under Rule 4.9(a) (*mark the applicable check-boxes; at least one must be marked*):

Regional Patent

☒ AP **ARIPO Patent**: GH Ghana, GM Gambia, KE Kenya, LS Lesotho, MW Malawi, SD Sudan, SL Sierra Leone, SZ Swaziland, TZ United Republic of Tanzania, UG Uganda, ZW Zimbabwe and any other State which is a Contracting State of the Harare Protocol and of the PCT

☒ EA **Eurasian Patent**: AM Armenia, AZ Azerbaijan, BY Belarus, KG Kyrgyzstan, KZ Kazakstan, MD Republic of Moldova, RU Russian Federation, TJ Tajikistan, TM Turkmenistan, and any other State which is a Contracting State of the Eurasian Patent Convention and of the PCT

☒ EP **European Patent**: AT Austria, BE Belgium, CH and LI Switzerland and Liechtenstein, CY Cyprus, DE Germany, DK Denmark, ES Spain, FI Finland, FR France, GB United Kingdom, GR Greece, IE Ireland, IT Italy, LU Luxembourg, MC Monaco, NL Netherlands, PT Portugal, SE Sweden, and any other State which is a Contracting State of the European Patent Convention and of the PCT

☒ OA **OAPI Patent**: BF Burkina Faso, BJ Benin, CF Central African Republic, CG Congo, CI Côte d'Ivoire, CM Cameroon, GA Gabon, GN Guinea, GW Guinea-Bissau, ML Mali, MR Mauritania, NE Niger, SN Senagal, TD Chad, TG Togo, and any other State which is a member State of OAPI and a Contracting State of the PCT (*if other kind of protection or treatment desired, specify on dotted line*).....

National Patent (*if other kind of protection or treatment desired, specify on dotted line*):

☒ AE	United Arab Emirates	☒ LS	Lesotho
☒ AG	Antigua and Barbuda	☒ LT	Lithuania
☒ AL	Albania	☒ LU	Luxembourg
☒ AM	Armenia	☒ LV	Latvia
☒ AT	Austria	☒ MA	Morocco
☒ AU	Australia	☒ MD	Republic of Moldova
☒ AZ	Azerbaijan	☒ MG	Madagascar
☒ BA	Bosnia and Herzegovina	☒ MK	The former Yugoslav Republic of Macedonia
☒ BB	Barbados		
☒ BG	Bulgaria	☒ MN	Mongolia
☒ BR	Brazil	☒ MW	Malawi
☒ BY	Belarus	☒ MX	Mexico
☒ CA	Canada	☒ NO	Norway
☒ CH and LI	Switzerland and Liechtenstein	☒ NZ	New Zealand
☒ CN	China	☒ PL	Poland
☒ CR	Costa Rica	☒ PT	Portugal
☒ CU	Cuba	☒ RO	Romania
☒ CZ	Czech Republic	☒ RU	Russian Federation
☒ DE	Germany	☒ SD	Sudan
☒ DK	Denmark	☒ SE	Sweden
☒ DM	Dominica	☒ SG	Singapore
☒ DZ	Algeria	☒ SI	Slovenia
☒ EE	Estonia	☒ SK	Slovakia
☒ ES	Spain	☒ SL	Sierra Leone
☒ FI	Finland	☒ TJ	Tajikistan
☒ GB	United Kingdom	☒ TM	Turkmenistan
☒ GD	Grenada	☒ TR	Turkey
☒ GE	Georgia	☒ TT	Trinidad and Tobago
☒ GH	Ghana	☒ TZ	United Republic of Tanzania
☒ GM	Gambia	☒ UA	Ukraine
☒ HR	Croatia	☒ UG	Uganda
☒ HU	Hungary	☒ US	United States of America
☒ ID	Indonesia		
☒ IL	Israel	☒ UZ	Uzbekistan
☒ IN	India	☒ VN	Viet Nam
☒ IS	Iceland	☒ YU	Yugoslavia
☒ JP	Japan	☒ ZA	South Africa
☒ KE	Kenya	☒ ZW	Zimbabwe
☒ KG	Kyrgyzstan		
☒ KP	Democratic People's Republic of Korea		
☒ KR	Republic of Korea		
☒ KZ	Kazakstan		
☒ LC	Saint Lucia		
☒ LK	Sri Lanka		
☒ LR	Liberia		

Check-boxes reserved for designating States (for the purposes of a national patent) which have become party to the PCT after issuance of this sheet:

☐ .....

☐ .....

Precautionary Designation Statement: In addition to the designations made above, the applicant also makes under Rule 4.9(b) all other designations which would be permitted under the PCT except any designation(s) indicated in the Supplemental Box as being excluded from the scope of this statement. The applicant declares that those additional designations are subject to confirmation and that any designation which is not confirmed before the expiration of 15 months from the priority date is to be regarded as withdrawn by the applicant at the expiration of that time limit. (*Confirmation (including fees) must reach the receiving office within the 15-month time limit.*)

Box No. VI PRIORITY CLAIM ☐ Further priority claims are indicated in the Supplemental Box

Filing date of earlier application *(day/month/year)*	Number of earlier application	Where earlier application is:		
		national application country	regional application:* regional Office	international application: receiving Office
item (1) 02 June 1999 (02.06.99)	99201745.9	EP		
item (2)				—
item (3)				

☐ The receiving Office is hereby requested to prepare and transmit to the International Bureau a certified copy of the earlier application(s) *(only if the earlier application was filed with the Office which for the purposes of the present international application is the receiving Office)* identified above as item(s): _____

* *Where the earlier application is an ARIPO application, it is mandatory to indicate in the Supplemental Box at least one country party to the Paris Convention for the Protection of Industrial Property for which that earlier application was filed (Rule 4.10(b)(ii)). See Supplemental Box.*

Box No. VII INTERNATIONAL SEARCHING AUTHORITY

Choice of International Searching Authority (ISA) *(if two or more International Searching Authorities are competent to carry out the international search, indicate the Authority chosen; the two-letter code may be used):*

ISA / EPO

Request to use results of earlier search; reference to that search *(if an earlier search has been carried out by or requested from the International Searching Authority):*

Date *(day/month/year)* Number Country (or regional Office)

Box No. VIII CHECK LIST; LANGUAGE OF FILING

This international application contains the following **number of sheets**:

request	:	5
description (excluding sequence listing part)	:	69
claims	:	10
abstract	:	1
drawings	:	11
sequence listing part of description	:	15
Total number of sheets	:	~~96~~ 111

This international application is **accompanied** by the item(s) marked below:

1. ☐ fee calculation sheet
2. ☐ separate signed power of attorney
3. ☐ copy of general power of attorney; reference number, if any:
4. ☐ statement explaining lack of signature
5. ☐ priority document(s) identified in Box No. VI as item(s):
6. ☐ translation of international application into (language):
7. ☐ separate indications concerning deposited microorganisms or other biological material
8. ☐ nucleoitide and/or amino acid sequence listing in computer readable form
9. ☒ other *(specify)*: Paper copy of sequence listing

Figure of the drawings which should accompany the abstract: 1

Language of filing of the international application: ENGLISH

Box No. IX SIGNATURE OF APPLICANT OR AGENT

Next to each signature, indicate the name of the person signing and the capacity in which the person signs (if such capacity is not obvious from reading the request).

Konrad A. Sechley
Patent Agent
Gowling, Strathy & Henderson

For receiving Office use only

1. Date of actual receipt of the purported international application: 02 JUN 2000 (02.06.00)
2. Drawings:
   ☒ received:
   ☐ not received:
3. Corrected date of actual receipt due to later but timely received papers or drawings completing the purported international application:
4. Date of timely receipt of the required corrections under PCT Article 11(2):
5. International Searching Authority (if two or more are competent): ISA /
6. ☒ Transmittal of search copy delayed until search fee is paid

For International Bureau use only

Date of receipt of the record copy by the International Bureau:

See Notes to the request form

[redacted]ENT COOPERATION TREATY [redacted]

From the
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

To:
SECHLEY, Konrad A. et al.
Gowling Lafleur Henderson LLP
Suite 2600
160 Elgin Street
Ottawa, Ontario K1P 1C3
CANADA

NOTIFICATION OF RECEIPT OF DEMAND BY COMPETENT INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

(PCT Rules 59.3(e) and 61.1(b), first sentence and Administrative Instructions, Section 601(a))

Date of mailing *(day/month/year)*: 25.01.01

Applicant's or agent's file reference: 08-887547WO

IMPORTANT NOTIFICATION

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/CA 00/00642	02/06/2000	02/06/1999

Applicant: PLANT RESEARCH INTERNATIONAL et al.

1. The applicant is hereby **notified** that this International Preliminary Examining Authority considers the following date as the date of receipt of the demand for international preliminary examination of the international application:

13/12/2000

2. This date of receipt is:

- [X] the actual date of receipt of the demand by this Authority (Rule 61.1(b)).
- [ ] the actual date of receipt of the demand on behalf of this Authority (Rule 59.3(e)).
- [ ] the date on which this Authority has, in response to the invitation to correct defects in the demand (Form PCT/IPEA/404), received the required corrections.

3. [ ] **ATTENTION:** That date of receipt is **AFTER** the expiration of 19 months from the priority date. Consequently, the election(s) made in the demand does (do) not have the effect of postponing the entry into the national phase until 30 months from the priority date (or later in some Offices) (Article 39(1)). Therefore, the acts for entry into the national phase must be performed within 20 months from the priority date (or later in some Offices) (Article 22). For details, see the *PCT Applicant's Guide*, Volume II.

[ ] *(If applicable)* This notification confirms the information given by telephone, facsimile transmission or in person on: _____

NB: Please see copy of page 2 of the Demand: The second entry of Kim Boutilier has been cancelled ex officio.

4. Only where paragraph 3 applies, a copy of this notification has been sent to the International Bureau.

Name and mailing address of the IPEA/	Authorized officer
European Patent Office D-80298 Munich Tel. (+49-89) 2399-0, Tx: 523656 epmu d Fax: (+49-89) 2399-4465	VON KEMPIS B G M Tel. (+49-89) 2399-8577

EUROPÄISCHES PATENTAMT · EUROPEAN PATENT OFFICE · OFFICE EUROPÉEN DES BREVETS

PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

To:

Commissioner
US Department of Commerce
United States Patent and Trademark Office, PCT
2011 South Clark Place Room CP2/5C24
Arlington, VA 22202
ETATS-UNIS D'AMERIQUE

in its capacity as elected Office

Date of mailing (day/month/year) 09 February 2001 (09.02.01)	
International application No. PCT/CA00/00642	**Applicant's or agent's file reference** 08-887547WO
International filing date (day/month/year) 02 June 2000 (02.06.00)	**Priority date** (day/month/year) 02 June 1999 (02.06.99)
Applicant BOUTILER, Kim et al	

1. The designated Office is hereby notified of its election made:

[X] in the demand filed with the International Preliminary Examining Authority on:

13 December 2000 (13.12.00)

[ ] in a notice effecting later election filed with the International Bureau on:

2. The election [X] was

[ ] was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau f WIPO **34, chemin des Colombettes** **1211 G neva 20, Switzerland**	*Authorized officer* A. Karkachi
Facsimile No.: (41-22) 740.14.35	Telephone No.: (41-22) 338.83.38

ID PFMAL3P4 standard; DNA; 113880 BP.
XX
AC AL008970; AL139179;
XX
SV AL008970.3
XX
DT 18-NOV-1997 (Rel. 53, Created)
DT 29-APR-2000 (Rel. 63, Last updated, Version 12)
XX
DE Plasmodium falciparum MAL3P4
XX
KW HTG.
XX
OS Plasmodium falciparum (malaria parasite P. falciparum)
OC Eukaryota; Alveolata; Apicomplexa; Haemosporida; Plasmodium.
XX
RN [1]
RP 1-113880
RA Hamlin N., Bowman S., Churcher C., Lawson D., Quail M., Barrell B.;
RT ;
RL Unpublished.
XX
RN [2]
RP 1-113880
RA Bowman S., Lawson D., Basham D., Brown D., Chillingworth T., Churcher C.M.,
RA Craig A., Davies R.M, Devlin K., Feltwell T., Gentles S., Gwilliam R.,
RA Hamlin N., Harris D., Holroyd S., Hornsby T., Horrocks P., Jagels K.,
RA Jassal B., Kyes S., McLean J., Moule S., Mungall K., Murphy L., Oliver K.,
RA Quail M.A., Rajandream M.-A., Rutter S., Skelton J., Squares R.,
RA Squares S., Sulston J.E., Whitehead S., Woodward J.R., Newbold C.,
RA Barrell B.G.;
RT "The complete nucleotide sequence of chromosome 3 of Plasmodium falciparum
RT ";
RL Nature 400:532-538(1999).
XX
RN [3]
RP 1-113880
RA Lawson D., Bowman S., Barrell B.;
RT ;
RL Submitted (18-NOV-1998) to the EMBL/GenBank/DDBJ databases.
RL P.falciparum Genome Sequencing Consortium, The Sanger Centre, Wellcome
RL Trust Genome Campus, Hinxton, Cambridge CB10 1SA, UK
XX
DR SPTREMBL; O77337; O77337.
DR SPTREMBL; O77338; O77338.
DR SPTREMBL; O77339; O77339.
DR SPTREMBL; O77340; O77340.
DR SPTREMBL; O77341; O77341.
DR SPTREMBL; O77342; O77342.
DR SPTREMBL; O77343; O77343.
DR SPTREMBL; O77344; O77344.
DR SPTREMBL; O77345; O77345.
DR SPTREMBL; O77346; O77346.
DR SPTREMBL; O77347; O77347.
DR SPTREMBL; O77348; O77348.
DR SPTREMBL; O77349; O77349.
DR SPTREMBL; O77350; O77350.
DR SPTREMBL; O77351; O77351.
DR SPTREMBL; O77353; O77353.
DR SPTREMBL; O77354; O77354.
DR SPTREMBL; O77355; O77355.
DR SPTREMBL; O77356; O77356.
DR SPTREMBL; O77357; O77357.
DR SPTREMBL; O77358; O77358.
DR SPTREMBL; O77359; O77359.
DR SPTREMBL; O77360; O77360.
DR SPTREMBL; O77361; O77361.
DR SPTREMBL; O77363; O77363.
DR SPTREMBL; O77364; O77364.
DR SPTREMBL; O77365; O77365.
XX

CC For more informa[redacted] about this sequence or the M[redacted]ria Project,
CC see http://www.sa[redacted]er.ac.uk/Projects/P_falciparum.
XX

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>>EM_INV:PFMAL3P4 AL008970 [redacted]SMODIUM FALCIPARUM MAL3P4    ([redacted]4 nt)
 initn: 240 init1: 161 opt: [redacted]5  Z-score: 209.7  bits: 58.2 [redacted]: 3.6e-07
 58.841% identity (61.859% ungapped) in 328 nt overlap (9-326:102415-102736)

                                        10        20        30
Seq.6-                                  TCTCAAACTCATCCATCTGATTTTAATAACAGTTTTTT
                                        :::: : : :: ::  :  :: ::::::
EM_INV TGTTCATTTTGTTCATATTGTTCACATTTTTCATTTTTTTCATATT--TTTCATTTTTTT
       102390    102400    102410    102420    102430    102440

       40        50        60        70        80        90
Seq.6- CTTCTTTTTC-TTTTGTTGTTTTTTACCACTTTTCTTTCTTTTTCTCATTTTCTAC-TTA
       : : :::::: :::: ::  :::::  :: ::::::   ::::: :::::::::: ::
EM_INV CATATTTTTCATTTTTTTCATTTTTTTCA-TTTTCTACATTTTTTTCATTTTCTACATTT
         102450    102460    102470    102480    102490    102500

        100       110       120       130       140       150
Seq.6- CTTCCAGATTTTTCATTTTCCTATTTTTGGTCACACGCT-CTTGTCAGTTGTAGATATCT
        :: ::  :: : ::::::  :  ::::  :::   .:: : : :   :  ::  : ::
EM_INV TTTTCATTTTCTACATTTTTTTCATTTTTTTCATTTTCTACATTTTTTTCATATTTTTCA
         102510    102520    102530    102540    102550    102560

         160       170       180       190       200           210
Seq.6- TCATCTACAGGTGTTTCCTTTTATTTTCAGATGGAATCTCAATCT-ACAGGTG----TTT
       :  ::::::  : :::: :::  ::::  : ::::   : ::: : :    ::     : :
EM_INV TTTTCTACATTTTTTTCATTTCCTTTT--GTTGGACAATAAATATGATCAATGAAAATAT
         102570    102580      102590    102600    102610

              220       230       240       250       260       270
Seq.6- CTCACTTCAATAAATTACGGCCCCCAAAAAATTTAGTTTTTGTATTTACAAGAAACATAG
        :   :::  :: : :  :  :   :: : : ::: : :::::::: :::: :   : :
EM_INV TTGGATTCCTTACAATGAGCGCATAAATATAATTATTATTTGTATTAACAATAGTTAAAT
  102620    102630    102640    102650    102660    102670

              280         290       300       310       320
Seq.6- CATAATATGATACAT--ATGGTTTTGAAGTACTGTTTTTTACACAAAACTTTGATTATAA
        :: :: :  ::: :   :   ::  :: ::     :: :   ::::: : : ::: :
EM_INV AATTAT-TATTACTTTCCTTCCTTATAACTAAAAAATTATTTCCAAAAATATTATTGTTT
  102680     102690    102700    102710    102720    102730

      330       340       350       360       370       380
Seq.6- AACCTCAGCCGTTCTTTCGTATTTAGAATTTAAACGCATGCAATGAAGTCATTCGTGAAT

EM_INV TCTTTTTTCTTGTCTCTGGATGTTGTTAATAAATTATCTAAGAGTAATTCTTTTCCCACT
   102740    102750    102760    102770    102780    102790
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(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 208 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Met Thr Ser Ser Val Ile Val Ala Gly Ala Gly Asp Lys Asn Asn Gly
1 5 10 15

Ile Val Val Gln Gln Gln Pro Pro Cys Val Ala Arg Glu Gln Asp Gln
20 25 30

Tyr Met Pro Ile Ala Asn Val Ile Arg Ile Met Arg Lys Thr Leu Pro
35 40 45

Ser His Ala Lys Ile Ser Asp Asp Ala Lys Glu Thr Ile Gln Glu Cys
50 55 60

Val Ser Glu Tyr Ile Ser Phe Val Thr Gly Glu Ala Asn Glu Arg Cys
65 70 75 80

Gln Arg Glu Gln Arg Lys Thr Ile Thr Ala Glu Asp Ile Leu Trp Ala
85 90 95

Met Ser Lys Leu Gly Phe Asp Asn Tyr Val Asp Pro Leu Thr Val Phe
100 105 110

Ile Asn Arg Tyr Arg Glu Ile Glu Thr Asp Arg Gly Ser Ala Leu Arg
115 120 125

Gly Glu Pro Pro Ser Leu Arg Gln Thr Tyr Gly Gly Asn Gly Ile Gly
130 135 140

Phe His Gly Pro Ser His Gly Leu Pro Pro Pro Gly Pro Tyr Gly Tyr
145 150 155 160

Gly Met Leu Asp Gln Ser Met Val Met Gly Gly Gly Arg Tyr Tyr Gln
165 170 175

Asn Gly Ser Ser Gly Gln Asp Glu Ser Ser Val Gly Gly Gly Ser Ser
180 185 190

Ser Ser Ile Asn Gly Met Pro Ala Phe Asp His Tyr Gly Gln Tyr Lys
195 200 205

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 3395 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

AGATCCAAAA CAGGTCATGG ACTGGGCCGT AAACTCTATC CAAAATTCTT CATGTTTTTC 60

CATCTTTCAA AAATCTTTAT CCACCATTCC ATTACTAGGG TGTTGGTTTT ATTTTATTTG 120

TTGATTAATT ATGTATTAGA AAATGTAAAG CAATATTCAA TTGTAACATG CATCATCTAA 180

CACCAATATC TTGTACTAAC CTTTTGTAAT TTTCCTATAA ACATTTTAAA AGGCTAATTT 240

AAATAAAAAT TACAATAAAC GTGATAACTC ACTTTCGTAA CGCATATTTA TTCAAATATA 300

CCAAAATTTA CCATTTTAAG TAAGAGAATC TTTTTAAAAT TAATTTTCAA TTTCATTAAT 360

TAAGAAACAA AGAATTTACT GAAACCTATA TTTTATTAAA TTTTAATAAA ATATATGACT 420

AAAATAACGT CACGTGAATC TTTCTCAGCC GTTCGATAAT CGAATACTTT ATTGACTAAG 480

TATTTATTTA GAAAATTTTA AACAACACTT AATTTCTAGA AACAAAGAGA GCCTCATATG 540

TATAAAAATC TTCTTCTTAT CTTTCTTTCT TTCTTAATAG TCTTTATTTT TACTTAATTA 600

CTTTGGTAAT TTGTGAAAAA CACAACCAAT GAGAGAAGAG CAGTTTGACT GGCCACATAG 660

CCAATGAGAC AAGCCAATGG GAAAGAGATA TAGAGACCTC GTAAGAACCG CTCCTTTGCC 720

ATTTGTATCA TCTCTCTATA AAACCACTCA ACCATCAACC TNTCTTTGCA TGCAACAAAT 780

CACTCAAATA ATTATTTTAT AAAGAACAAA AAAAAAAAGA CGGCAGAGAA ACAATGGAAC 840

GTGGAGCTCC CTTCTCTCAC TATCAGCTAC CCAAATCCAT CTCTGGTAAT CTAAGTGGCT 900

ATTTGTATAC AGTATATACT TGCCTCCATG TATATTTATA TTCTCGTGAA AAATTGGAGA 960

CATGCTTTAT GAATTTTATG AGACTTTGCA ACAACGAACG AGATGCTTTC TCTCTAGAAA 1020

TTTAAATTTA GATTTGTGAA GGTTTTGGGA ATGGCCCGGA GAAGACGATT TTATATATAC 1080

ATGCATGCAA GAGTTTGATA TGTATATTGT TTCATCATGG CTGAGTCAAA GTTTTATCCA 1140

AATATTTCCA TGGTGTGGTA TTAGTTAAAC AAATCTCTCG TATGTGTCAT TGAATATACC 1200

CGTGCATGTA CCAGGAATGT TTTTGATTCT AAAAACGTTT TTTTCTTTGT TGTAACGGTT 1260

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TATTGAGTCA TACCTTCGTC AACATAAATT AATTGATGTT CTTCTCCAAA TTTTGAGTTT 1680

TTGGTTTTTC TAATAATCTT AACGAAAGCT TTTTGGTATA CATGTAAAAC GTAACGGCAA 1740

GAATCTGAAC AGTCTACTCA ACGGGGTCCA TAAGTCTAGA ATGTAGACCC CACAAACTTA 1800

CTCTTATCTT ATTGGTCCGT AACTAAGAAC GTGTCCCTCT GATTCTCTTG TTTTCTTCTA 1860

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CCTAAAGATT TCAATTTCTC TCTGTATTTT CTATGAACAG AATTGAACTT GGACCAGCAC 1980

AGCAACAACC CAACCCCAAT GACCAGCTCA GTCATAGTAG CCGGCGCCGG TGACAAGAAC 2040

AATGGTATCG TGGTCCAGCA GCAACCACCA TGTGTGGCTC GTGAGCAAGA CCAATACATG 2100

CCAATCGCAA ACGTCATAAG AATCATGCGT AAAACCTTAC CGTCTCACGC CAAAATCTCT 2160

GACGACGCCA AAGAAACGAT TCAAGAATGT GTCTCCGAGT ACATCAGCTT CGTGACCGGT 2220

GAAGCCAACG AGCGTTGCCA ACGTGAGCAA CGTAAGACCA TAACTGCTGA AGATATCCTT 2280

TGGGCTATGA GCAAGCTTGG GTTCGATAAC TACGTGGACC CCCTCACCGT GTTCATTAAC 2340

CGGTACCGTG AGATAGAGAC CGATCGTGGT TCTGCACTTA GAGGTGAGCC ACCGTCGTTG 2400

AGACAAACCT ATGGAGGAAA TGGTATTGGG TTTCACGGCC CATCTCATGG CCTACCTCCT 2460

CCGGGTCCTT ATGGTTATGG TATGTTGGAC CAATCCATGG TTATGGGAGG TGGTCGGTAC 2520

TACCAAAACG GGTCGTCGGG TCAAGATGAA TCCAGTGTTG GTGGTGGCTC TTCGTCTTCC 2580

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AATAACATTT CTATATAATG TTGCTTCTTT AAGGAAAAGT GTTGTATGTC AATACTTTAT 2760

GAGAAACTGA TTTATATATG CAAATGATTG AATCCAAACT GTTTTGTGGA TTAAACTCTA 2820

TGCAACATTA TATATTTACA TGATCTAAAG GTTTTGTAAT TCAAAAGCTG TCATAGTTAG 2880

AAGATAACTA AACATTGTAG TAACCAAGTT TAATTTACTT TTTTGAGTTT ACATAACTAA 2940

CCAAGCCAAA AGGTTATAAA ATCTAAATTC GTTGAGTTGT CAAACTTCTG AAGATTGCTA 3000

TCCTCTTTGA GTTGCTTTCT TTTGGGTGCT TGAGTTTCAT TAGGCTGAGC TGACTCGTTG 3060

CTCTCTAGTC TTTCATCTCT GTCTTTTCCA AGGATTCATA ACGTTGGTCG CTCTCTGTTT 3120

CTGCCTACAC TTCTTCAAGG GATCATTACT GAGGCTAAGA GTTAAAGACC TGAACCATGG 3180

TTTTCTGTAA CTGGTTCAAG TTCATTCTCC GGTTATTGTG TGGTTATCTT TCGGTTAGAT 3240

TGAAACCCAT ATGTTTGCTC TGTTTCTTCT AGTTCCAAGT TTAATTTCCG GTTATTGTTT 3300

GGCTTTTTAA AAGTTTTTAA GGTCTATTCT ATGTAAAGAC TATTCTACGT ACGTACATTT 3360

ATCGCAAAAT TGAAAGATTA TAAAAAAAAT TGAAA 3395

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 7560 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

AATTNACCCT CACTAAAGGG AACAAAAGCT GGGTACCGGG CCCCCCCTCG AGGTCGACGG 60

TATCGATAAG CTTGATATCG AATTCGTGGC CATTAGACCC ATAACTATAT GACGATGTTA 120

AAGAGAAAAT AAATCATAAA TAAAATAAGA GTCCTTATCA ATAAACCTAA TTGGCTAATT 180

TCAACCTCAA AGAGTAGTAG GAACAGGTAA GGTGAAGCCA AACAGCTCCT TTTACAGTTG 240

GACCACTAGA GCTGATCTGG CATACAAAGT ATGCTTATTG GGCTGTCACG GCCCATCCGC 300

AAAATGTCGT TGGTTACGAA GCATCCACGA CATAGACGGT GCCACATGTT AGAAAAGTGT 360

TTCGGCGATC AAGATTGTGT CCACATCATT AGACGTCTGA ACTGTCCACG TGTCTATCAA 420

AGCTGGCGTC AAACATTACG TTTTCGTCGT TTGCGCCTCC TAGTTCACAC GTGCAACGAA 480

CGCGTGCGAC GTATCAAAAT TGTTAATTTT AGCCATGTAT AAAGAATATC TACAAAATTA 540

ACCTCAGGAA TATTTTTGTT TTTTCAATTG AGGCCATAAT ATACNTNCCG ATNGAAAAAT 600

TTTNCANCAT ATCNCTAATA TCAAAAAATT ATGATGTTAG TAAACGTAAA AAATTTACAC 660

AAAATAANTT TCACAAAACT TANNGGGGAA ATTGGAACAA ANAAAAGACT GGTGAGTGAT 720

AAGCGATGAT GGCCGGTGAA TCAGGTAGCC GTCCTACAAC GTGGTTGATT TTGAGCAAAC 780

TCCTATCTAC TCTTCACACT ATTGGAAATC CCAAAATGTC GTCACACCAT AATAATGTGA 840

ATTTTGTTAT GGAATTTGAG GGAAACAGTA GATATATGTT TCAACCAGTG AAAGTTACCC 900

TCCTTTGGAC ATATCTACGA NAGTAGAAAG TAGAAACATT CACTAAACGT GACAACTTTA 960

TAAATTTTCT TTTTGTAACT TTTCTTTAGA TTTATTTACG ANAAGAGAAA TATAAACGTC 1020

ATGCTAATAA AAAATGCATT ATTTTCTACC ATCTAGCTAG AATATTGATC AAGTCTTCAC 1080

GTTTTTTGTT TATCTCTTCT CTCATAGGCA TGTCCACAAA AGGGTAAGTT TTACTGGTTC 1140

AAAATATTGC ATGAGTACTA CTAAGCTCGT ATAGTTTGAT CTTACTATCA TTGCGATGAG 1200

GGTTGTTAGT TTGGAAGAAA TAAGGATTTA TGCAAATGGT AATCATTATG TCTGCTATTT 1260

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ATACACGAGA GAAGCTCAGA TATTCACGTA ACGATGTTTC ATCTCTTCTC ATTGAGGAAA 1380

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AAACTGTTCC ATCTCTTATT ATATATAGTT TGGTTATTTA AGTAATTAGA TGCATCATAA 1500

TCTTTTTTTC TGCCAGTTGT AATGCAGATA AAAATATATT GGTTGTTCTA AGGATTGTTC 1560

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ATAATGATAA AACTAAGATT TTTAGTTAAA TTTAATAAAG AGCTTACGAG CTACAATTAA 1680

TTAGAAATGG TTGCTCAGAA ATCAGAATAC TATATATGAA AAAAGAAGTT GGTATACTTG 1740

AAAAAAGAAA AAACTACTTG AAAAGATGGT AAAAGATATA GAACGAGTAT ATATCTTACT 1800

CAAGCACGAT AGAAGTTTGT ATCAAAACAT TGCGTTCCAA ACCAATGTTT GAAGATGGTC 1860

AAAGGTGCTA CTCATGATGT GGTGCGAAGA AGCTTACGAA AAATTCTGCA ATGAGAGATA 1920

ACTTTATGGG CTGCTTGTTC AATATATTGA AAATCATGGT AGACAACACC AAACTCTCCT 1980

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TGTATTATCC TATCTCTTTA GCTATCCTTT CCTTGCTCTT GCTCCACCTG CATGTGGCCT 2160

CTTTATTTAT AATCTCTCTA GATTCTGCTA AAGATGTNTG TTCAAAATGG TTTATCTTTA 2220

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AGATTTCATA CTGATGATAA CTAAAATAAT CTTATATGCG TAAGATTATT TTAGTTCTAA 2340

ACTTCATTTT GAAATGAGAG GTCATTGGCC AGGAAAGATT CAATATTGGT TCTTTGTTAA 2400

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TCTTAATAGT CTTTATTTTT ACTTAATTAC TTTGGTAATT TGTGAAAAAC ACAACCAATG 3060

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CCATCAACCT NTCTTTGCAT GCAACAAATC ACTCAAATAA TTATTTTATA AAGAACAAAA 3240

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CAAATCCATC TCTGGTAATC TAAGTGGCTA TTTGTATACA GTATATACTT GCCTCCATGT 3360

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CAACGAACGA GATGCTTTCT CTCTAGAAAT TTAAATTTAG ATTTGTGAAG GTTTTGGGAA 3480

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AAGTCTAGAA TGTAGACCCC ACAAACTTAC TCTTATCTTA TTGGTCCGTA ACTAAGAACG 4260

TGTCCCTCTG ATTCTCTTGT TTTCTTCTAA TTAATTCGTA TCCTACAAAT TTAATTATCA 4320

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CCAGTGTTGG TGGTGGCTCT TCGTCTTCCA TTAACGGAAT GCCGGCTTTT GACCATTATG 5040

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TTGAGTTGTC AAACTTCTGA AGATTGCTAT CCTCTTTGAG TTGCTTTCTT TTGGGTGCTT 5460

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GTTCCAAGTT TAATTTCCGG TTATTGTTTG GCTTTTTAAA AGTTTTTAAG GTCTATTCTA 5760

TGTAAAGACT ATTCTACGTA CGTACATTTA TCGCAAAATT GAAAGATTAT AAAAAAAATT 5820

GAAAGATCCA AAGGAAACCA ATAGATTAAA CTAAAATGTA GTATCCTTTT TATCATTTTA 5880

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TAAACTAAAG GGCGTAAATG CAAGCTTGTT ATGCGTTATT GAAAACATTA CCTCTAAATT 6060

AAATAGCCCA ATATTGAAAA CCTTAAGCTT CTTTGATCCC CTTAACTTGT TTGTCCACCA 6120

AGTATTAGTT CATCTCTTAA CACGGCAACT CGAAACGGCA CAATGGACAA ACATGGTCTT 6180

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CTATTTCTCC CTTTTCAAAC TCCTCCAAAC AAATTGTGCA CACACTGGCG TCAGAGTTGG 6300

ATTTCTTCTT ATTATTATAT ACTTTCCTTG CCAAACGGTC AACCACAAAC TTATTTGCCG 6360

GTCTAATTAA CTCGATATTA TTGGTGGTCT CATCAAACGA GTCAATCCGA GGAGGAGGTG 6420

GAACAATGAC TTTACAGTAC ATGTAAACTA ACGTAGCACA AACTGAAGAG TCTACCATAG 6480

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TGACACCTTC GTAGTCATTG TACGAGAGAA CGAAATTTGG TGGAAGACTA ATTGATGTGT 6660

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CATATTGTAC TAAGAAAAGT AGAAAACCAG ACTTTTTAAT ATGTTAGATT TTAATTGGGT 7200

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CTTTTTTTTT TATATATCGA CTACCATAAA ACTTTAACGA TTAATATTTT TGGATAATAA 7380

GCGATCGTTT TGAGGCGTCC CAATTTTTTT TGTTTCTTTT TATATGAGAA ATGGGTTTAA 7440

GAAAAACTGC AATTTTGTCC ATAAAGCTAG TCAGAATTCC TGCAGCCCGG GGGATCCACT 7500

AGTTCTAGAG CGGCCGCCAC CGCGGTGGAG CTCCAATTCG CCCTATAGTG AGTCGTATTA 7560

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 7 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

Met Pro Ile Ala Asn Val Ile
1 5

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 12 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

Ile Gln Glu Cys Val Ser Glu Tyr Ile Ser Phe Val
1 5 10

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 25 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

GGAATTCAGC AACAACCCAA CCCCA 25

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

GCTCTAGACA TACAACACTT TTCCTTA 27

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

ATGACCAGCT CAGTCATAGT AGC 23

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

GCCACACATG GTGGTTGCTG CTG 23

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23 base pairs
(B) TYPE: nucleic acid

(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

GAGATAGAGA CCGATCGTGG TTC 23

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

TCACTTATAC TGACCATAAT GGTC 24

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

GCATAGATGC ACTCGAAATC AGCC 24

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

GCTTGGTAAT AATTGTCATT AG 22

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

CTAAAAACAT CTACGGTTCA

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

TTTGTGGTTG ACCGTTTGGC 20

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 7 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

Leu Pro Ile Ala Asn Val Ala
1 5

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 12 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

Met Gln Glu Cys Val Ser Glu Phe Ile Ser Phe Val
1 5 10

WHAT IS CLAIMED IS:

1. An isolated nucleic acid molecule comprising a *LEC1* polynucleotide sequence, which polynucleotide sequence specifically hybridizes to SEQ. ID. No. 1 under stringent conditions.

2. The isolated nucleic acid molecule of claim 1, wherein the *LEC1* polynucleotide is between about 100 nucleotides and about 630 nucleotides in length.

3. The isolated nucleic acid molecule of claim 1, wherein the *LEC1* polynucleotide is SEQ. ID. No. 1.

4. The isolated nucleic acid molecule of claim 1, wherein the *LEC1* polynucleotide encodes a LEC1 polypeptide of between about 50 and about 210 amino acids.

5. The isolated nucleic acid molecule of claim 4, wherein the LEC1 polypeptide has an amino acid sequence as shown in SEQ. ID. No. 2.

6. The isolated nucleic acid molecule of claim 1, further comprising a plant promoter operably linked to the *LEC1* polynucleotide.

7. The isolated nucleic acid molecule of claim 6, wherein the plant promoter is from a *LEC1* gene.

8. The isolated nucleic acid of claim 7, wherein the *LEC1* gene is as shown in SEQ. ID. No. 3.

9. The isolated nucleic acid of claim 7, wherein the *LEC1* gene is as shown in SEQ. ID. No. 4.

10. The isolated nucleic acid of claim 7, wherein the *LEC1* polynucleotide is linked to the promoter in an antisense orientation.

11. An isolated nucleic acid molecule comprising a *LEC1* polynucleotide sequence, which polynucleotide sequence encodes LEC1 polypeptide of between about 50 and about 210 amino acids.

12. The isolated nucleic acid of claim 10, wherein the LEC1 polypeptide has an amino acid sequence as shown in SEQ. ID. No. 2.

13. A transgenic plant comprising an expression cassette containing a plant promoter operably linked to a heterologous *LEC1* polynucleotide that specifically hybridizes to SEQ. ID. No. 1 under stringent conditions.

14. The transgenic plant of claim 12, wherein the heterologous *LEC1* polynucleotide encodes a LEC1 polypeptide.

15. The transgenic plant of claim 13, wherein the LEC1 polypeptide is SEQ. ID. No. 2.

16. The transgenic plant of claim 12, wherein the heterologous *LEC1* polynucleotide is linked to the promoter in an antisense orientation.

17. The transgenic plant of claim 12, wherein the plant promoter is from a *LEC1* gene.

18. The transgenic plant of claim 16, wherein the *LEC1* gene is as shown in SEQ. ID. No. 3.

19. The transgenic plant of claim 12, which is a member of the genus *Brassica*.

20. A method of modulating seed development in a plant, the method comprising introducing into the plant an expression cassette containing a plant promoter

operably linked to a heterologous *LEC1* polynucleotide that specifically hybridizes to SEQ. ID. No. 1 under stringent conditions.

21. The method of claim 19, wherein the heterologous *LEC1* polynucleotide encodes a LEC1 polypeptide.

22. The method of claim 20, wherein the LEC1 polypeptide has an amino acid sequence as shown in SEQ. ID. No. 2.

23. The method of claim 19, wherein the heterologous *LEC1* polynucleotide is linked to the promoter in an antisense orientation.

24. The method of claim 19, wherein the heterologous *LEC1* polynucleotide is SEQ. ID. No. 1.

25. The method of claim 19, wherein the plant promoter is from a *LEC1* gene.

26. The method of claim 19, wherein the *LEC1* gene is as shown in SEQ. ID. No. 3.

27. The method of claim 19, wherein the plant is a member of the genus *Brassica*.

28. The method of claim 19, wherein the expression cassette is introduced into the plant through a sexual cross.

29. An isolated nucleic acid molecule comprising a plant promoter that specifically hybridizes to a polynucleotide sequence consisting of nucleotides 1 to 1998 of SEQ. ID. No. 3.

30. The isolated nucleic acid molecule of claim 28, wherein the plant promoter sequence consists essentially of nucleotides 1 to 1998 of SEQ. ID. No. 3.

31. The isolated nucleic acid molecule of claim 28, wherein the plant promoter sequence is a subsequence of SEQ. ID. No. 4.

32. The isolated nucleic acid molecule of claim 28, further comprising a polynucleotide sequence operably linked to the plant promoter sequence.

33. The isolated nucleic acid of claim 30, wherein the polynucleotide sequence operably linked to the plant promoter sequence encodes a desired polypeptide.

34. The isolated nucleic acid molecule of claim 28, wherein the polynucleotide sequence is linked to the promoter in an antisense orientation.

35. A transgenic plant comprising an expression cassette containing a *LEC1* promoter operably linked to a heterologous polynucleotide sequence, wherein the *LEC1* promoter specifically hybridizes to SEQ. ID. No. 3 under stringent conditions.

36. The transgenic plant of claim 33, wherein the polynucleotide sequence encodes a desired polypeptide.

37. The transgenic plant of claim 33, wherein the heterologous polynucleotide sequence is linked to the *LEC1* promoter in an antisense orientation.

38. The transgenic plant of claim 33, wherein the *LEC1* promoter is as shown in SEQ. ID. No. 3.

39. The transgenic plant of claim 33, which is a member of the genus *Brassica*.

40. A method of targeting expression of a polynucleotide to a seed, the method comprising introducing into a plant an expression cassette containing a *LEC1* promoter operably linked to a heterologous polynucleotide sequence, wherein the *LEC1* promoter specifically hybridizes to a polynucleotide sequence consisting of nucleotides 1 to -1998 of SEQ. ID. No. 3.

41. The method of claim 38, wherein the heterologous polynucleotide sequence encodes a desired polypeptide.

42. The method of claim 38, wherein the heterologous polynucleotide sequence is linked to the promoter in an antisense orientation.

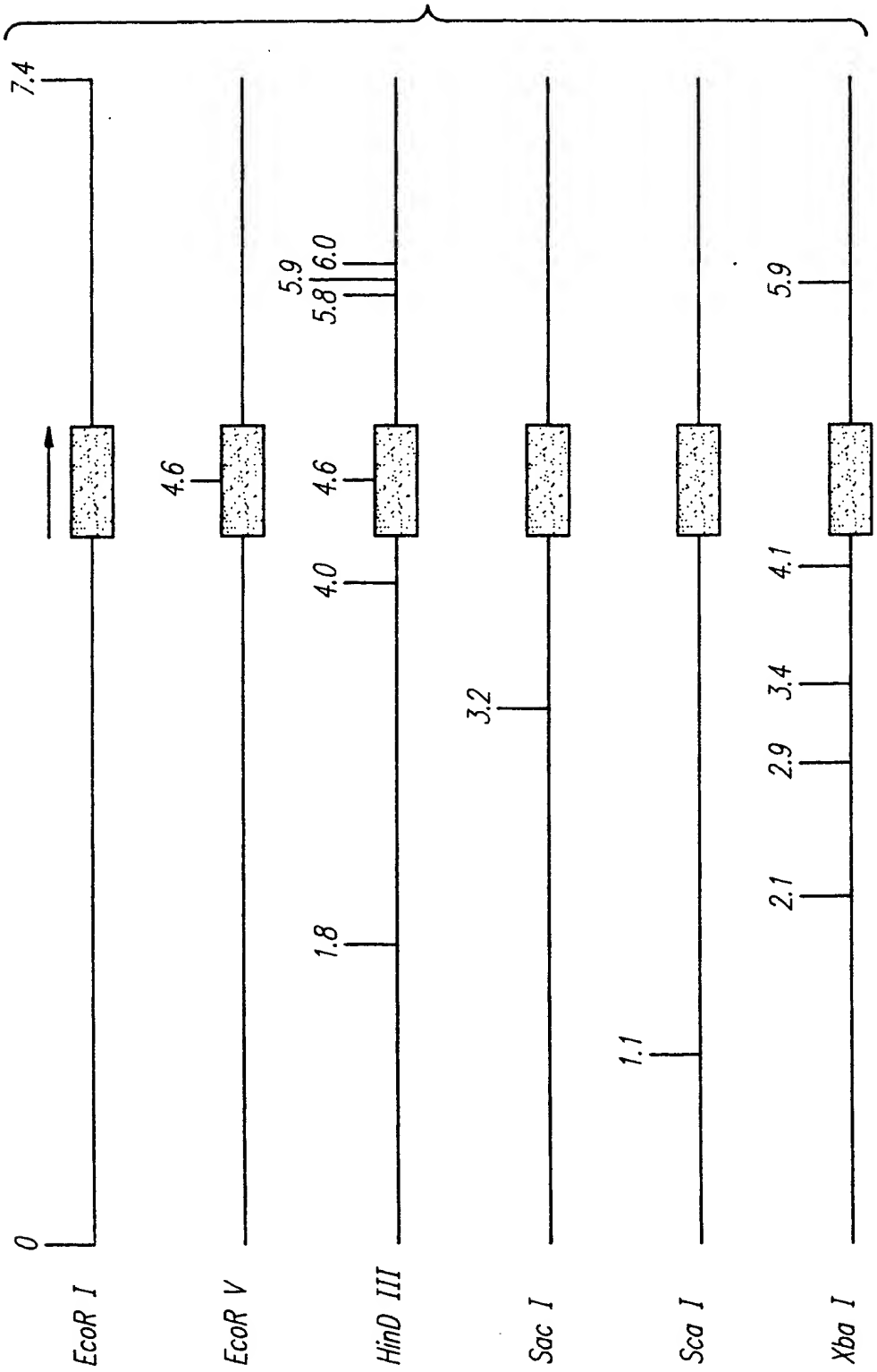

FIG. 1

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US98/02998

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) : C12N 15/00, 15/09, 15/29, 15/82; A01H 1/00, 3/00, 5/00
US CL : 536/23.6, 24.1, 24.5; 435/172.3, 252.2, 320.1; 800/205

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 536/23.6, 24.1, 24.5; 435/172.3, 252.2, 320.1; 800/205

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Agricola, Wpids, STN, Biosis, NCBI, APS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	MEINKE, D.W. A homeotic Mutant of *Arabidopsis thaliana* with Leafy Cotyledons. Science. 04 December 1992, Vol. 258, pages 1647-1650. See entire reference.	1-42
Y	HECK et al. AGL15, a MADS Domain Protein Expressed in Developing Embryos. The Plant Cell. August 1995, Vol. 7, pages 1271-1282, see entire reference.	1-42
Y	VALVEKENS et al. *Agrobacterium tumefaciens*-Mediated Transformation of *Arabidopsis thaliana* Root Explants by Using Kanamycin Selection. Proc. Natl. Acad. Sci., USA, August 1988, Vol. 85, pages 5536-5540, see entire reference	13-28, 35-42

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"B"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search	Date of mailing of the international search report
06 APRIL 1998	09 JUN 1998
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer Ousama M-Faiz Zaghmout Telephone No. (703) 308-0196

Form PCT/ISA/210 (second sheet)(July 1992)*

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US98/02998

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	CHAN et al. Novel Gene Expression System for Plant Cells Based on Induction of α-Amylase Promoter by Carbohydrate Starvation. The Journal of Biological Chemistry. 01 July 1994, Vol. 269, No. 26, pages 17635-17641, see entire reference.	7-10, 17, 25, 29-42
Y	SHIMADA et al. Antisense Regulation of the Rice Waxy Gene Expression Using a PCR-Amplified Fragment of the Rice Genome Reduces the Amylose Content in Grain Starch. Theor. Appl. Genet. 1993, Vol. 86, pages 665-672, see entire content.	10, 12, 14, 16-19, 21, 23-28, 34, 37, 42

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US98/02998

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

Please See Extra Sheet.

1. ☒ **As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.**

2. ☐ **As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.**

3. ☐ **As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:**

4. ☐ **No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:**

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING
This ISA found multiple inventions as follows:

This application contains the following inventions or groups of inventions which are not linked as to form a single inventive concept under PCT Rule 13.1.

Group I. Claims 1-6, 11, 13, 15, 20, and 22 are drawn to nucleic acid molecule comprising a LEC1 gene, vectors containing it in sense orientation, methods for their use to transform plants, and the resultant transgenic plants, classified in class 800, subclass 205 for example.

Group II. Claims 7-10, 17, 25, 29- 42 are drawn to LEC1 promoters, methods for their use in constructs to transform plants, and the resultant transgenic plants, classified in class 536, subclass 24.1 for example.

Group III. Claims 10, 12, 14, 16-19, 21, 23-28, 34, 37, and 42 are drawn to vectors containing sequences in antisense orientation, methods for their use to transform plants, and the resultant transgenic plants, classified in class 536, subclass 24.5 for example.

The inventions listed as groups I, II and III do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons: The invention of group I, drawn to a first process of making a first product, involves making the construct and transforming plants not required by the groups II and III.

The invention of group I involves the usage of coding sequences of LEC1 in the sense orientation, not required by groups II-III.

The invention of group II involves the LEC1 promoter sequences and their usage not required by groups I and III, and a non-LEC1 structural gene not required by group I.

The invention of group III involves the usage of coding sequences of LEC1 or other genes in the antisense orientation, and methods for evaluation of gene suppression, not required by groups I-II.

LEAFY COTYLEDON1 GENES AND THEIR USES

FIELD OF THE INVENTION

The present invention is directed to plant genetic engineering. In particular, it relates to new embryo-specific genes useful in improving agronomically important plants.

BACKGROUND OF THE INVENTION

Embryogenesis in higher plants is a critical stage of the plant life cycle in which the primary organs are established. Embryo development can be separated into two main phases: the early phase in which the primary body organization of the embryo is laid down and the late phase which involves maturation, desiccation and dormancy. In the early phase, the symmetry of the embryo changes from radial to bilateral, giving rise to a hypocotyl with a shoot meristem surrounded by the two cotyledonary primordia at the apical pole and a root meristem at the basal pole. In the late phase, during maturation the embryo achieves its maximum size and the seed accumulates storage proteins and lipids. Maturation is ended by the desiccation stage in which the seed water content decreases rapidly and the embryo passes into metabolic quiescent state. Dormancy ends with seed germination, and development continues from the shoot and the root meristem regions.

The precise regulatory mechanisms which control cell and organ differentiation during the initial phase of embryogenesis are largely unknown. The plant hormone abscisic acid (ABA) is thought to play a role during late embryogenesis, mainly in the maturation stage by inhibiting germination during embryogenesis (Black, M. (1991). In Abscisic Acid: Physiology and Biochemistry, W. J. Davies and H. G. Jones, eds. (Oxford: Bios Scientific Publishers Ltd.), pp. 99-124) Koornneef, M., and Karssen, C. M. (1994). In Arabidopsis, E. M. Meyerowitz and C. R. Sommerville, eds. (Cold Spring Harbor: Cold Spring Harbor Laboratory Press), pp. 313-334). Mutations which effect seed development and are ABA insensitive have been identified in *Arabidopsis* and maize. The ABA insensitive (abi3) mutant of *Arabidopsis* and the viviparous1 (vp1) mutant of maize are detected mainly during late embryogenesis (McCarty, *et al.*, (1989) *Plant Cell* 1, 523-532 and Parcy *et al.*, (1994) *Plant Cell* 6, 1567-1582). Both the *VP1* gene and the *ABI3* genes have been isolated and were

found to share conserved regions (Giraudat, J. (1995) *Current Opinion in Cell Biology* 7:232-238 and McCarty, D. R. (1995). *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 46:71-93). The *VP1* gene has been shown to function as a transcription activator (McCarty, *et al.*, (1991) *Cell* 66:895-906). It has been suggested that *ABI3* has a similar function.

Another class of embryo defective mutants involves three genes: *LEAFY COTYLEDON1* and *2* (*LEC1*, *LEC2*) and *FUSCA3 (FUS3)*. These genes are thought to play a central role in late embryogenesis (Baumlein, *et al.* (1994) *Plant J.* 6:379-387; Meinke, D. W. (1992) *Science* 258:1647-1650; Meinke *et al.*, *Plant Cell* 6:1049-1064; West *et al.*, (1994) *Plant Cell* 6:1731-1745). Like the abi3 mutant, leafy cotyledon-type mutants are defective in late embryogenesis. In these mutants, seed morphology is altered, the shoot meristem is activated early, storage proteins are lacking and developing cotyledons accumulate anthocyanin. As with abi3 mutants, they are desiccation intolerant and therefore die during late embryogenesis. Nevertheless, the immature mutants embryos can be rescued to give rise to mature and fertile plants. However, unlike abi3 when the immature mutants germinate they exhibit trichomes on the adaxial surface of the cotyledon. Trichomes are normally present only on leaves, stems and sepals, not cotyledons. Therefore, it is thought that the leafy cotyledon type genes have a role in specifying cotyledon identity during embryo development.

Among the above mutants, the *lec1* mutant exhibits the most extreme phenotype during embryogenesis. For example, the maturation and postgermination programs are active simultaneously in the *lec1* mutant (West et al., 1994), suggesting a critical role for *LEC1* in gene regulation during late embryogenesis.

In spite of the recent progress in defining the genetic control of embryo development, further progress is required in the identification and analysis of genes expressed specifically in the embryo and seed. Characterization of such genes would allow for the genetic engineering plants with a variety of desirable traits. For instance, modulation of the expression of genes which control embryo development may be used to alter traits such as accumulation of storage proteins in leaves and cotyledons. Alternatively, promoters from embryo or seed-specific genes can be used to direct expression of desirable heterologous genes to the embryo or seed. The present invention addresses these and other needs.

SUMMARY OF THE INVENTION

The present invention is based, in part, on the isolation and characterization of *LEC1* genes. The invention provides isolated nucleic acid molecules comprising a *LEC1* polynucleotide sequence, typically about 630 nucleotides in length, which specifically hybridizes to SEQ. ID. No. 1 under stringent conditions. The *LEC1* polynucleotides of the invention can encode a LEC1 polypeptide of about 210 amino acids, typically as shown in SEQ. ID. No. 2.

The nucleic acids of the invention may also comprise expression cassettes containing a plant promoter operably linked to the *LEC1* polynucleotide. In some embodiments, the promoter is from a *LEC1* gene, for instance, as shown in SEQ. ID. No. 3. The *LEC1* polynucleotide may be linked to the promoter in a sense or antisense orientation.

The invention also provides transgenic plants comprising an expression cassette containing a plant promoter operably linked to a heterologous *LEC1* polynucleotide. The *LEC1* may encode a LEC1 polypeptide or may be linked to the promoter in an antisense orientation. The plant promoter may be from any number of sources, including a *LEC1* gene, such a as that shown in SEQ. ID. No. 3 or SEQ. ID. No. 4. The transgenic plant can be any desired plant but is often a member of the genus *Brassica*.

Methods of modulating seed development in a plants are also provided. The methods comprise introducing into a plant an expression cassette containing a plant promoter operably linked to a heterologous *LEC1* polynucleotide. The *LEC1* may encode a LEC1 polypeptide or may be linked to the promoter in an antisense orientation. The expression cassette can be introduced into the plant by any number of means known in the art, including through a sexual cross.

The invention further provides expression cassettes containing promoter sequences from *LEC1* genes. The promoters of the invention can be characterized by their ability to specifically hybridizes to a polynucleotide sequence consisting of nucleotides 1 to -1998 of SEQ. ID. No. 3. The promoters of the invention can be operably linked to a variety of nucleic acids, whose expression is to be targeted to embryos or seeds. Transgenic plants comprising the expression cassettes are also provided.

The promoters of the invention can be used in methods of targeting expression of a desired polynucleotide to seeds. The methods comprise introducing into a plant an

expression cassette containing a *LEC1* promoter operably linked to a heterologous polynucleotide sequence.

Definitions

The phrase "nucleic acid" refers to a single or double-stranded polymer of deoxyribonucleotide or ribonucleotide bases read from the 5' to the 3' end. Nucleic acids may also include modified nucleotides that permit correct read through by a polymerase and do not alter expression of a polypeptide encoded by that nucleic acid.

The phrase "polynucleotide sequence" or "nucleic acid sequence" includes both the sense and antisense strands as either individual single strands or in the duplex. It includes, but is not limited to, self-replicating plasmids, chromosomal sequences, and infectious polymers of DNA or RNA.

The phrase "nucleic acid sequence encoding" refers to a nucleic acid which directs the expression of a specific protein or peptide. The nucleic acid sequences include both the DNA strand sequence that is transcribed into RNA and the RNA sequence that is translated into protein. The nucleic acid sequences include both the full length nucleic acid sequences as well as non-full length sequences derived from the full length sequences. It should be further understood that the sequence includes the degenerate codons of the native sequence or sequences which may be introduced to provide codon preference in a specific host cell.

The term "promoter" refers to a region or sequence determinants located upstream or downstream from the start of transcription and which are involved in recognition and binding of RNA polymerase and other proteins to initiate transcription. A "plant promoter" is a promoter capable of initiating transcription in plant cells. Such promoters need not be of plant origin, for example, promoters derived from plant viruses, such as the CaMV35S promoter, can be used in the present invention.

The term "plant" includes whole plants, plant organs (e.g., leaves, stems, flowers, roots, etc.), seeds and plant cells and progeny of same. The class of plants which can be used in the method of the invention is generally as broad as the class of higher plants amenable to transformation techniques, including both monocotyledonous and dicotyledonous plants, as well as certain lower plants such as algae. It includes plants of a variety of ploidy levels, including polyploid, diploid and haploid.

A polynucleotide sequence is "heterologous to" an organism or a second polynucleotide sequence if it originates from a foreign species, or, if from the same species, is modified from its original form. For example, a promoter operably linked to a heterologous coding sequence refers to a coding sequence from a species different from that from which the promoter was derived, or, if from the same species, a coding sequence which is different from any naturally occurring allelic variants. As defined here, a modified *LEC1* coding sequence which is heterologous to an operably linked *LEC1* promoter does not include the T-DNA insertional mutants as described in West *et al.*, *The Plant Cell* 6:1731-1745 (1994).

A polynucleotide "exogenous to" an individual plant is a polynucleotide which is introduced into the plant by any means other than by a sexual cross. Examples of means by which this can be accomplished are described below, and include *Agrobacterium*-mediated transformation, biolistic methods, electroporation, *in planta* techniques, and the like. Such a plant containing the exogenous nucleic acid is referred to here as an R_1 generation transgenic plant. Transgenic plants which arise from sexual cross or by selfing are descendants of such a plant.

As used herein an "embryo-specific gene" or "seed specific gene" is a gene that is preferentially expressed during embryo development in a plant. For purposes of this disclosure, embryo development begins with the first cell divisions in the zygote and continues through the late phase of embryo development (characterized by maturation, desiccation, dormancy), and ends with the production of a mature and desiccated seed. Embryo-specific genes can be further classified as "early phase-specific" and "late phase-specific". Early phase-specific genes are those expressed in embryos up to the end of embryo morphogenesis. Late phase-specific genes are those expressed from maturation through to production of a mature and desiccated seed.

A "*LEC1* polynucleotide" is a nucleic acid sequence comprising (or consisting of) a coding region of about 100 to about 900 nucleotides, sometimes from about 300 to about 630 nucleotides, which hybridizes to SEQ. ID. No. 1 under stringent conditions (as defined below), or which encodes a LEC1 polypeptide. *LEC1* polynucleotides can also be identified by their ability to hybridize under low stringency conditions (*e.g.*, Tm -40°C) to nucleic acid probes having a sequence from position 1 to 81 in SEQ. ID. NO. 1 or from position 355 to 627 in SEQ. ID. NO. 1.

A "promoter from a *LEC1* gene" or "*LEC1* promoter" will typically be about 500 to about 2000 nucleotides in length, usually from about 750 to 1500. An exemplary promoter sequence is shown as nucleotides 1-1998 of SEQ. ID. No. 3. A *LEC1* promoter can also be identified by its ability to direct expression in all, or essentially all, proglobular embryonic cells, as well as cotyledons and axes of a late embryo.

A "LEC1 polypeptide" is a sequence of about 50 to about 210, sometimes 100 to 150, amino acid residues encoded by a *LEC1* polynucleotide. A full length LEC1 polypeptide and fragments containing a CCAAT binding factor (CBF) domain can act as a subunit of a protein capable of acting as a transcription factor in plant cells. LEC1 polypeptides are often distinguished by the presence of a sequence which is required for binding the nucleotide sequence: CCAAT. In particular, a short region of seven residues (MPIANVI) at residues 34-40 of SEQ. ID No. 3 shows a high degree of similarity to a region that has been shown to required for binding the CCAAT box. Similarly, residues 61-72 of SEQ. ID No. 3 (IQECVSEYISFV) is nearly identical to a region that contains a subunit interaction domain (Xing, *et al.*, (1993) *EMBO J.* 12:4647-4655).

As used herein, a homolog of a particular embryo-specific gene (*e.g.*, SEQ. ID. No. 1) is a second gene in the same plant type or in a different plant type, which has a polynucleotide sequence of at least 50 contiguous nucleotides which are substantially identical (determined as described below) to a sequence in the first gene. It is believed that, in general, homologs share a common evolutionary past.

A "polynucleotide sequence from" a particular embryo-specific gene is a subsequence or full length polynucleotide sequence of an embryo-specific gene which, when present in a transgenic plant, has the desired effect, for example, inhibiting expression of the endogenous gene driving expression of an heterologous polynucleotide. A full length sequence of a particular gene disclosed here may contain about 95%, usually at least about 98% of an entire sequence shown in the Sequence Listing, below.

In the case of both expression of transgenes and inhibition of endogenous genes (e.g., by antisense, or sense suppression) one of skill will recognize that the inserted polynucleotide sequence need not be identical and may be "substantially identical" to a sequence of the gene from which it was derived. As explained below, these variants are specifically covered by this term.

In the case where the inserted polynucleotide sequence is transcribed and translated to produce a functional polypeptide, one of skill will recognize that because of codon degeneracy a number of polynucleotide sequences will encode the same polypeptide. These variants are specifically covered by the term "polynucleotide sequence from" a particular embryo-specific gene, such as *LEC1*. In addition, the term specifically includes sequences (*e.g.*, full length sequences) substantially identical (determined as described below) with a *LEC1* gene sequence and that encode proteins that retain the function of a LEC1 polypeptide.

In the case of polynucleotides used to inhibit expression of an endogenous gene, the introduced sequence need not be perfectly identical to a sequence of the target endogenous gene. The introduced polynucleotide sequence will typically be at least substantially identical (as determined below) to the target endogenous sequence.

Two nucleic acid sequences or polypeptides are said to be "identical" if the sequence of nucleotides or amino acid residues, respectively, in the two sequences is the same when aligned for maximum correspondence as described below. The term "complementary to" is used herein to mean that the sequence is complementary to all or a portion of a reference polynucleotide sequence.

Optimal alignment of sequences for comparison may be conducted by the local homology algorithm of Smith and Waterman *Add. APL. Math.* 2:482 (1981), by the homology alignment algorithm of Needle man and Wunsch *J. Mol. Biol.* 48:443 (1970), by the search for similarity method of Pearson and Lipman *Proc. Natl. Acad. Sci. (U.S.A.)* 85: 2444 (1988), by computerized implementations of these algorithms (GAP, BESTFIT, BLAST, FASTA, and TFASTA in the Wisconsin Genetics Software Package, Genetics Computer Group (GCG), 575 Science Dr., Madison, WI), or by inspection.

"Percentage of sequence identity" is determined by comparing two optimally aligned sequences over a comparison window, wherein the portion of the polynucleotide sequence in the comparison window may comprise additions or deletions (i.e., gaps) as compared to the reference sequence (which does not comprise additions or deletions) for optimal alignment of the two sequences. The percentage is calculated by determining the number of positions at which the identical nucleic acid base or amino acid residue occurs in both sequences to yield the number of matched positions, dividing the number of matched

positions by the total number of positions in the window of comparison and multiplying the result by 100 to yield the percentage of sequence identity.

The term "substantial identity" of polynucleotide sequences means that a polynucleotide comprises a sequence that has at least 80% sequence identity, preferably at least 85%, more preferably at least 90% and most preferably at least 95%, compared to a reference sequence using the programs described above (preferably BLAST) using standard parameters. One of skill will recognize that these values can be appropriately adjusted to determine corresponding identity of proteins encoded by two nucleotide sequences by taking into account codon degeneracy, amino acid similarity, reading frame positioning and the like. Substantial identity of amino acid sequences for these purposes normally means sequence identity of at least 40%, preferably at least 60%, more preferably at least 90%, and most preferably at least 95%. Polypeptides which are "substantially similar" share sequences as noted above except that residue positions which are not identical may differ by conservative amino acid changes. Conservative amino acid substitutions refer to the interchangeability of residues having similar side chains. For example, a group of amino acids having aliphatic side chains is glycine, alanine, valine, leucine, and isoleucine; a group of amino acids having aliphatic-hydroxyl side chains is serine and threonine; a group of amino acids having amide-containing side chains is asparagine and glutamine; a group of amino acids having aromatic side chains is phenylalanine, tyrosine, and tryptophan; a group of amino acids having basic side chains is lysine, arginine, and histidine; and a group of amino acids having sulfur-containing side chains is cysteine and methionine. Preferred conservative amino acids substitution groups are: valine-leucine-isoleucine, phenylalanine-tyrosine, lysine-arginine, alanine-valine, aspartic acid-glutamic acid, and asparagine-glutamine.

Another indication that nucleotide sequences are substantially identical is if two molecules hybridize to each other, or a third nucleic acid, under stringent conditions. Stringent conditions are sequence dependent and will be different in different circumstances. Generally, stringent conditions are selected to be about 5° C lower than the thermal melting point (Tm) for the specific sequence at a defined ionic strength and pH. The Tm is the temperature (under defined ionic strength and pH) at which 50% of the target sequence hybridizes to a perfectly matched probe. Typically, stringent conditions will be those in which the salt concentration is about 0.02 molar at pH 7 and the temperature is at least about 60°C.

In the present invention, mRNA encoded by embryo-specific genes of the invention can be identified in Northern blots under stringent conditions using cDNAs of the invention or fragments of at least about 100 nucleotides. For the purposes of this disclosure, stringent conditions for such RNA-DNA hybridizations are those which include at least one wash in 0.2X SSC at 63°C for 20 minutes, or equivalent conditions. Genomic DNA or cDNA comprising genes of the invention can be identified using the same cDNAs (or fragments of at least about 100 nucleotides) under stringent conditions, which for purposes of this disclosure, include at least one wash (usually 2) in 0.2X SSC at a temperature of at least about 50°C, usually about 55°C, for 20 minutes, or equivalent conditions.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a restriction map of the 7.4 kb genomic wild-type fragment shown in SEQ. ID. No. 4.

DESCRIPTION OF THE PREFERRED EMBODIMENT

The present invention provides new embryo-specific genes useful in genetically engineering plants. Polynucleotide sequences from the genes of the invention can be used, for instance, to direct expression of desired heterologous genes in embryos (in the case of promoter sequences) or to modulate development of embryos or other organs (*e.g.*, by enhancing expression of the gene in a transgenic plant) In particular, the invention provides a new gene from *Arabidopsis* referred to here as *LEC1*. *LEC1* encodes polypeptides which subunits of a protein which acts as a transcription factor. Thus, modulation of the expression of this gene can be used to manipulate a number of useful traits, such as increasing or decreasing storage protein content in cotyledons or leaves.

Generally, the nomenclature and the laboratory procedures in recombinant DNA technology described below are those well known and commonly employed in the art. Standard techniques are used for cloning, DNA and RNA isolation, amplification and purification. Generally enzymatic reactions involving DNA ligase, DNA polymerase, restriction endonucleases and the like are performed according to the manufacturer's specifications. These techniques and various other techniques are generally performed according to Sambrook *et al.*, *Molecular Cloning - A Laboratory Manual*, 2nd. ed., Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, (1989).

Isolation of nucleic acids of the invention

The isolation of sequences from the genes of the invention may be accomplished by a number of techniques. For instance, oligonucleotide probes based on the sequences disclosed here can be used to identify the desired gene in a cDNA or genomic DNA library from a desired plant species. To construct genomic libraries, large segments of genomic DNA are generated by random fragmentation, e.g. using restriction endonucleases, and are ligated with vector DNA to form concatemers that can be packaged into the appropriate vector. To prepare a library of embryo-specific cDNAs, mRNA is isolated from embryos and a cDNA library which contains the gene transcripts is prepared from the mRNA.

The cDNA or genomic library can then be screened using a probe based upon the sequence of a cloned embryo-specific gene such as the polynucleotides disclosed here. Probes may be used to hybridize with genomic DNA or cDNA sequences to isolate homologous genes in the same or different plant species.

Alternatively, the nucleic acids of interest can be amplified from nucleic acid samples using amplification techniques. For instance, polymerase chain reaction (PCR) technology to amplify the sequences of the genes directly from mRNA, from cDNA, from genomic libraries or cDNA libraries. PCR and other *in vitro* amplification methods may also be useful, for example, to clone nucleic acid sequences that code for proteins to be expressed, to make nucleic acids to use as probes for detecting the presence of the desired mRNA in samples, for nucleic acid sequencing, or for other purposes.

Appropriate primers and probes for identifying embryo-specific genes from plant tissues are generated from comparisons of the sequences provided herein. For a general overview of PCR see *PCR Protocols: A Guide to Methods and Applications*. (Innis, M, Gelfand, D., Sninsky, J. and White, T., eds.), *Academic Press*, San Diego (1990). Appropriate primers for this purpose include, for instance: UP primer - 5' GGA ATT CAG CAA CAA CCC AAC CCC A 3" and LP primer - 5' LP primer - 5' GCT CTA GAC ATA CAA CAC TTT TCC TTA 3'. Alternatively, the following primer pairs can be used: 5' ATG ACC AGC TCA GTC ATA GTA GC 3' and 5' GCC ACA CAT GGT GGT TGC TGC TG 3' or 5' GAG ATA GAG ACC GAT CGT GGT TC 3' and 5' TCA CTT ATA CTG ACC ATA ATG GTC 3'. The amplifications conditions are typically as follows. Reaction components: 10 mM Tris-HCl, pH 8.3, 50 mM potassium chloride, 1.5 mM magnesium

chloride, 0.001% gelatin, 200 microM dATP, 200 microM dCTP, 200 microM dGTP, 200 microM dTTP, 0.4 microM primers, and 100 units per ml Taq polymerase. Program: 96 C for 3 min., 30 cycles of 96 C for 45 sec., 50 C for 60 sec., 72 for 60 sec, followed by 72 C for 5 min.

Polynucleotides may also be synthesized by well-known techniques as described in the technical literature. See, e.g., Carruthers *et al.*, *Cold Spring Harbor Symp. Quant. Biol.* 47:411-418 (1982), and Adams *et al.*, *J. Am. Chem. Soc.* 105:661 (1983). Double stranded DNA fragments may then be obtained either by synthesizing the complementary strand and annealing the strands together under appropriate conditions, or by adding the complementary strand using DNA polymerase with an appropriate primer sequence.

Use of nucleic acids of the invention to inhibit gene expression

The isolated sequences prepared as described herein, can be used to prepare expression cassettes useful in a number of techniques. For example, expression cassettes of the invention can be used to suppress endogenous *LEC1* gene expression. Inhibiting expression can be useful, for instance, in weed control (by transferring an inhibitory sequence to a weedy species and allowing it to be transmitted through sexual crosses) or to produce fruit with small and non-viable seed.

A number of methods can be used to inhibit gene expression in plants. For instance, antisense technology can be conveniently used. To accomplish this, a nucleic acid segment from the desired gene is cloned and operably linked to a promoter such that the antisense strand of RNA will be transcribed. The expression cassette is then transformed into plants and the antisense strand of RNA is produced. In plant cells, it has been suggested that antisense RNA inhibits gene expression by preventing the accumulation of mRNA which encodes the enzyme of interest, see, e.g., Sheehy et al , *Proc. Nat. Acad. Sci. USA*, 85:8805-8809 (1988), and Hiatt et al., U.S. Patent No. 4,801,340.

The nucleic acid segment to be introduced generally will be substantially identical to at least a portion of the endogenous embryo-specific gene or genes to be repressed. The sequence, however, need not be perfectly identical to inhibit expression. The vectors of the present invention can be designed such that the inhibitory effect applies to other proteins within a family of genes exhibiting homology or substantial homology to the target gene.

For antisense suppression, the introduced sequence also need not be full length relative to either the primary transcription product or fully processed mRNA. Generally, higher homology can be used to compensate for the use of a shorter sequence. Furthermore, the introduced sequence need not have the same intron or exon pattern, and homology of non-coding segments may be equally effective. Normally, a sequence of between about 30 or 40 nucleotides and about full length nucleotides should be used, though a sequence of at least about 100 nucleotides is preferred, a sequence of at least about 200 nucleotides is more preferred, and a sequence of at least about 500 nucleotides is especially preferred.

Catalytic RNA molecules or ribozymes can also be used to inhibit expression of embryo-specific genes. It is possible to design ribozymes that specifically pair with virtually any target RNA and cleave the phosphodiester backbone at a specific location, thereby functionally inactivating the target RNA. In carrying out this cleavage, the ribozyme is not itself altered, and is thus capable of recycling and cleaving other molecules, making it a true enzyme. The inclusion of ribozyme sequences within antisense RNAs confers RNA-cleaving activity upon them, thereby increasing the activity of the constructs.

A number of classes of ribozymes have been identified. One class of ribozymes is derived from a number of small circular RNAs which are capable of self-cleavage and replication in plants. The RNAs replicate either alone (viroid RNAs) or with a helper virus (satellite RNAs). Examples include RNAs from avocado sunblotch viroid and the satellite RNAs from tobacco ringspot virus, lucerne transient streak virus, velvet tobacco mottle virus, solanum nodiflorum mottle virus and subterranean clover mottle virus. The design and use of target RNA-specific ribozymes is described in Haseloff et al. *Nature*, 334:585-591 (1988).

Another method of suppression is sense suppression. Introduction of expression cassettes in which a nucleic acid is configured in the sense orientation with respect to the promoter has been shown to be an effective means by which to block the transcription of target genes. For an example of the use of this method to modulate expression of endogenous genes see, Napoli et al., *The Plant Cell* 2:279-289 (1990), and U.S. Patents Nos. 5,034,323, 5,231,020, and 5,283,184.

Generally, where inhibition of expression is desired, some transcription of the introduced sequence occurs. The effect may occur where the introduced sequence contains no coding sequence *per se*, but only intron or untranslated sequences homologous to

sequences present in the primary transcript of the endogenous sequence. The introduced sequence generally will be substantially identical to the endogenous sequence intended to be repressed. This minimal identity will typically be greater than about 65%, but a higher identity might exert a more effective repression of expression of the endogenous sequences. Substantially greater identity of more than about 80% is preferred, though about 95% to absolute identity would be most preferred. As with antisense regulation, the effect should apply to any other proteins within a similar family of genes exhibiting homology or substantial homology.

For sense suppression, the introduced sequence in the expression cassette, needing less than absolute identity, also need not be full length, relative to either the primary transcription product or fully processed mRNA. This may be preferred to avoid concurrent production of some plants which are overexpressers. A higher identity in a shorter than full length sequence compensates for a longer, less identical sequence. Furthermore, the introduced sequence need not have the same intron or exon pattern, and identity of non-coding segments will be equally effective. Normally, a sequence of the size ranges noted above for antisense regulation is used.

Another means of inhibiting LEC1 function in a plant is by creation of dominant negatives. In this approach, non-functional, mutant LEC1 polypeptides, which retain the ability to interact with wild-type subunits are introduced into a plant. Identification of residues that can be changed to create a dominant negative can be determined by published work examining interaction of different subunits of CBF homologs from different species (*see, e.g.*, Sinha *et al.*, (1995). *Proc. Natl. Acad. Sci. USA* 92:1624-1628.)

Use of nucleic acids of the invention to enhance gene expression

Isolated sequences prepared as described herein can also be used to prepare expression cassettes which enhance or increase endogenous *LEC1* gene expression. Where overexpression of a gene is desired, the desired gene from a different species may be used to decrease potential sense suppression effects. Enhanced expression of *LEC1* polynucleotides is useful, for example, to increase storage protein content in plant tissues. Such techniques may be particularly useful for improving the nutritional value of plant tissues.

One of skill will recognize that the polypeptides encoded by the genes of the invention, like other proteins, have different domains which perform different functions.

Thus, the gene sequences need not be full length, so long as the desired functional domain of the protein is expressed. As explained above, LEC1 polypeptides share sequences with CBF proteins. The DNA binding activity, and, therefore, transcription activation function, of LEC1 polypeptides is thought to be modulated by a short region of seven residues (MPIANVI) at residues 34-40 of SEQ. ID No. 2. Thus, the polypeptides of the invention will often retain these sequences. Modified protein chains can also be readily designed utilizing various recombinant DNA techniques well known to those skilled in the art and described for instance, in Sambrook *et al., supra*. Hydroxylamine can also be used to introduce single base mutations into the coding region of the gene (Sikorski, *et al.*, (1991). *Meth. Enzymol.* 194: 302-318). For example, the chains can vary from the naturally occurring sequence at the primary structure level by amino acid substitutions, additions, deletions, and the like. These modifications can be used in a number of combinations to produce the final modified protein chain.

Desired modified LEC1 polypeptides can be identified using assays to screen for the presence or absence of wild type LEC1 activity. Such assays can be based on the ability of the LEC1 protein to functionally complement the hap3 mutation in yeast. As noted above, it has been shown that homologs from different species functionally interact with yeast subunits of the CBF. (Sinha, *et al.*, (1995). *Proc. Natl. Acad. Sci. USA* 92:1624-1628); *see, also*, Becker, *et al.*, (1991). *Proc. Natl. Acad. Sci. USA* 88: 1968-1972). The reporter for this screen can be any of a number of standard reporter genes such as the lacZ gene encoding β-galactosidase that is fused with the regulatory DNA sequences and promoter of the yeast CYC1 gene. This promoter is regulated by the yeast CBF.

A plasmid containing the LEC1 cDNA clone is mutagenized *in vitro* according to techniques well known in the art. The cDNA inserts are excised from the plasmid and inserted into the cloning site of a yeast expression vector such as pYES2 (Invitrogen). The plasmid is introduced into hap3- yeast containing a lacZ reporter that is regulated by the yeast CBF such as pLG265UP1-lacZ (Guarente, *et al.*, (1984) *Cell* 36: 317-321). Transformants are then selected and a filter assay is used to test colonies for β-galactosidase activity. After confirming the results of activity assays, immunochemical tests using a LEC1 antibody are performed on yeast lines that lack β-galactosidase activity to identify those that produce stable LEC1 protein but lack activity. The mutant LEC1 genes are then cloned from the yeast and their nucleotide sequence determined to identify the nature of the lesions.

In other embodiments, the promoters derived from the *LEC1* genes of the invention can be used to drive expression of heterologous genes in an embryo-specific or seed-specific manner, such that desired gene products are present in the embryo, seed, or fruit. Suitable structural genes that could be used for this purpose include genes encoding proteins useful in increasing the nutritional value of seed or fruit. Examples include genes encoding enzymes involved in the biosynthesis of antioxidants such as vitamin A, vitamin C, vitamin E and melatonin. Other suitable genes encoding proteins involved in modification of fatty acids, or in the biosynthesis of lipids, proteins, and carbohydrates. Still other genes can be those encoding proteins involved in auxin and auxin analog biosynthesis for increasing fruit size, genes encoding pharmaceutically useful compounds, and genes encoding plant resistance products to combat fungal or other infections of the seed.

Typically, desired promoters are identified by analyzing the 5' sequences of a genomic clone corresponding to the embryo-specific genes described here. Sequences characteristic of promoter sequences can be used to identify the promoter. Sequences controlling eukaryotic gene expression have been extensively studied. For instance, promoter sequence elements include the TATA box consensus sequence (TATAAT), which is usually 20 to 30 base pairs upstream of the transcription start site. In most instances the TATA box is required for accurate transcription initiation. In plants, further upstream from the TATA box, at positions -80 to -100, there is typically a promoter element with a series of adenines surrounding the trinucleotide G (or T) N G. J. Messing et al., in *Genetic Engineering in Plants*, pp. 221-227 (Kosage, Meredith and Hollaender, eds. (1983)).

A number of methods are known to those of skill in the art for identifying and characterizing promoter regions in plant genomic DNA (*see, e.g.*, Jordano, *et al.*, *Plant Cell*, 1: 855-866 (1989); Bustos, *et al.*, *Plant Cell*, 1:839-854 (1989); Green, *et al.*, *EMBO J.* 7, 4035-4044 (1988); Meier, *et al.*, *Plant Cell*, 3, 309-316 (1991); and Zhang, *et al.*, *Plant Physiology* 110: 1069-1079 (1996)).

Preparation of recombinant vectors

To use isolated sequences in the above techniques, recombinant DNA vectors suitable for transformation of plant cells are prepared. Techniques for

transforming a wide variety of higher plant species are well known and described in the technical and scientific literature. See, for example, Weising et al. *Ann. Rev. Genet.* 22:421-477 (1988). A DNA sequence coding for the desired polypeptide, for example a cDNA sequence encoding a full length protein, will preferably be combined with transcriptional and translational initiation regulatory sequences which will direct the transcription of the sequence from the gene in the intended tissues of the transformed plant.

For example, for overexpression, a plant promoter fragment may be employed which will direct expression of the gene in all tissues of a regenerated plant. Such promoters are referred to herein as "constitutive" promoters and are active under most environmental conditions and states of development or cell differentiation. Examples of constitutive promoters include the cauliflower mosaic virus (CaMV) 35S transcription initiation region, the 1'- or 2'- promoter derived from T-DNA of *Agrobacterium tumafaciens*, and other transcription initiation regions from various plant genes known to those of skill.

Alternatively, the plant promoter may direct expression of the polynucleotide of the invention in a specific tissue (tissue-specific promoters) or may be otherwise under more precise environmental control (inducible promoters). Examples of tissue-specific promoters under developmental control include promoters that initiate transcription only in certain tissues, such as fruit, seeds, or flowers. As noted above, the promoters from the *LEC1* genes described here are particularly useful for directing gene expression so that a desired gene product is located in embryos or seeds. Other suitable promoters include those from genes encoding storage proteins or the lipid body membrane protein, oleosin. Examples of environmental conditions that may affect transcription by inducible promoters include anaerobic conditions, elevated temperature, or the presence of light.

If proper polypeptide expression is desired, a polyadenylation region at the 3'-end of the coding region should be included. The polyadenylation region can be derived from the natural gene, from a variety of other plant genes, or from T-DNA.

The vector comprising the sequences (*e.g.*, promoters or coding regions) from genes of the invention will typically comprise a marker gene which confers a selectable phenotype on plant cells. For example, the marker may encode biocide

resistance, particularly antibiotic resistance, such as resistance to kanamycin, G418, bleomycin, hygromycin, or herbicide resistance, such as resistance to chlorosluforon or Basta.

Production of transgenic plants

DNA constructs of the invention may be introduced into the genome of the desired plant host by a variety of conventional techniques. For example, the DNA construct may be introduced directly into the genomic DNA of the plant cell using techniques such as electroporation and microinjection of plant cell protoplasts, or the DNA constructs can be introduced directly to plant tissue using ballistic methods, such as DNA particle bombardment. Alternatively, the DNA constructs may be combined with suitable T-DNA flanking regions and introduced into a conventional *Agrobacterium tumefaciens* host vector. The virulence functions of the *Agrobacterium tumefaciens* host will direct the insertion of the construct and adjacent marker into the plant cell DNA when the cell is infected by the bacteria.

Microinjection techniques are known in the art and well described in the scientific and patent literature. The introduction of DNA constructs using polyethylene glycol precipitation is described in Paszkowski et al. *Embo J.* 3:2717-2722 (1984). Electroporation techniques are described in Fromm et al. *Proc. Natl. Acad. Sci. USA* 82:5824 (1985). Ballistic transformation techniques are described in Klein et al. *Nature* 327:70-73 (1987).

Agrobacterium tumefaciens-mediated transformation techniques, including disarming and use of binary vectors, are well described in the scientific literature. See, for example Horsch et al. *Science* 233:496-498 (1984), and Fraley et al. *Proc. Natl. Acad. Sci. USA* 80:4803 (1983).

Transformed plant cells which are derived by any of the above transformation techniques can be cultured to regenerate a whole plant which possesses the transformed genotype and thus the desired phenotype such as seedlessness. Such regeneration techniques rely on manipulation of certain phytohormones in a tissue culture growth medium, typically relying on a biocide and/or herbicide marker which has been introduced together with the desired nucleotide sequences. Plant regeneration from cultured protoplasts is described in Evans et al., *Protoplasts Isolation and Culture*,

Handbook of Plant Cell Culture, pp. 124-176, MacMillilan Publishing Company, New York, 1983; and Binding, *Regeneration of Plants, Plant Protoplasts*, pp. 21-73, CRC Press, Boca Raton, 1985. Regeneration can also be obtained from plant callus, explants, organs, or parts thereof. Such regeneration techniques are described generally in Klee et al. *Ann. Rev. of Plant Phys.* 38:467-486 (1987).

The nucleic acids of the invention can be used to confer desired traits on essentially any plant. Thus, the invention has use over a broad range of plants, including species from the genera *Asparagus, Atropa, Avena, Brassica, Citrus, Citrullus, Capsicum, Cucumis, Cucurbita, Daucus, Fragaria, Glycine, Gossypium, Helianthus, Heterocallis, Hordeum, Hyoscyamus, Lactuca, Linum, Lolium, Lycopersicon, Malus, Manihot, Majorana, Medicago, Nicotiana, Oryza, Panieum, Pannesetum, Persea, Pisum, Pyrus, Prunus, Raphanus, Secale, Senecio, Sinapis, Solanum, Sorghum, Trigonella, Triticum, Vitis, Vigna*, and, *Zea*. The *LEC1* genes of the invention are particularly useful in the production of transgenic plants in the genus *Brassica*. Examples include broccoli, cauliflower, brussel sprouts, canola, and the like.

One of skill will recognize that after the expression cassette is stably incorporated in transgenic plants and confirmed to be operable, it can be introduced into other plants by sexual crossing. Any of a number of standard breeding techniques can be used, depending upon the species to be crossed.

Example 1

This example describes the isolation and characterization of an exemplary *LEC1* gene.

Experimental Procedures

Plant Material

A *lec1-2* mutant was identified from a population of *Arabidopsis thaliana* ecotype Wassilewskija (Ws-O) lines mutagenized with T-DNA insertions as described before (West et al., 1994). The *abi3-3, fus3-3* and *lec1-1* mutants were generously provided by Peter McCourt, University of Toronto and David Meinke, Oklahoma State University. Wild type plants and mutants were grown under constant light at 22°C.

Double mutants were constructed by intercrossing the mutant lines *lec1-1, lec1-2, abi3-3, fus3-3*, and *lec2*. The genotype of the double mutants was verified through

backcrosses with each parental line. Double mutants were those who failed to complement both parent lines. Homozygous single and double mutants were generated by germinating intact seeds or dissected mature embryos before desiccation on basal media.

Isolation and Sequence analysis of Genomic and cDNA Clones

Genomic libraries of Ws-O wild type plants, *lec1-1* and *lec1-2* mutants were made in GEM11 vector according to the instructions of the manufacturer (Promega). Two silique-specific cDNA libraries (stages globular to heart and heart to young torpedo) were made in ZAPII vector (Stratagene).

The genomic library of *lec1-2* was screened using right and left T-DNA specific probes according to standard techniques. About 12 clones that cosegragate with the mutation, were isolated and purified and the entire DNAs were further labeled and used as probes to screen a southern blot containing wild type and *lec1-1* genomic DNA. One clone hybridized with plant DNA and was further analyzed. A 7.1 kb XhoI fragment containing the left border and the plant sequence flanking the T-DNA was subcloned into pBluescript-KS plasmid (Stratagene) to form ML7 and sequenced using a left border specific primer (5' GCATAGATGCACTCGAAATCAGCC 3') The T-DNA organization was partially verified using southern analysis with T-DNA left and right borders and PBR322 probes. The results suggested that the other end of the T-DNA is also composed of left border. This was confirmed by generating a PCR fragment using a genomic plant DNA primer (LP primer5' GCT CTA GAC ATA CAA CAC TTT TCC TTA 3') and a T-DNA left border specific primer (5' GCTTGGTAATAATTGTCATTAG 3') and sequencing.

The EcoRI insert of ML7 was used to screen a wild type genomic library. Two overlapping clones were purified and a 7.4 EcoRI genomic fragment from the wild type DNA region was subcloned into pBluescript-KS plasmid making WT74. This fragment was sequenced (SEQ. ID. No. 4) and was used to screen *lec1-1* genomic library and wild type silique-specific cDNA libraries. 8 clones from the *lec1-1* genomic library were identified and analyzed by restriction mapping.

From these clones the exact site of the deletion in *lec1-1* was mapped and sequenced by amplifying a Xbp PCR fragment using primers (H21 - 5' H21 - 5' CTA AAA ACA TCT ACG GTT CA 3'; H 17 - 5' TTT GTG GTT GAC CGT TTG GC 3') flanking the deletion region in *lec1-1* genomic DNA. Clones were isolated from both cDNA libraries and partially sequenced. The sequence of the cDNA clones and the wild type genomic clone

matched exactly, confirming that both derived from the same locus. All hybridizations were performed under stringent conditions with 32P random prime probes (Stratagene).

Sequencing was done using the automated dideoxy chain termination method (Applied Biosystems, Foster City, CA). Data base searches were performed at the National Center for Biotechnology Information by using the BLAST network service. Alignment of protein sequences was done using PILEUP program (Genetics Computer Group, Madison, WI)

DNA and RNA blot analysis

Genomic DNA was isolated from leaves by using the CTAB-containing buffer Dellaporta, *et al.*, (1983). *Plant Mol. Biol. Reporter* 1: 19-21. Two micrograms of DNA was digested with different restriction endonucleases, electrophoretically separated in 1% agarose gel, and transferred to a nylon membrane (Hybond N; Amersham).

Total RNA was prepared from siliques, two days old seedlings, stems, leaves, buds and roots. Poly(A)+ RNA was purified from total RNA by oligo(dT) cellulose chromatography, and two micrograms of each Poly(A)+ RNA samples were separated in 1% denatured formaldehyde-agarose gel. Hybridizations were done under stringent conditions unless it specifies otherwise. Radioactive probes were prepared as described above.

Complementation of lec1 mutants

A 3.4 kb BstyI fragment of genomic DNA (SEQ. ID. No. 3) containing sequences from 1.992 kb upstream of the ORF to a region 579 bp downstream from the poly A site was subcloned into the hygromycin resistant binary vector pBIB-Hyg. The LEC1 cDNA was placed under the control of the 35S promoter and the ocs polyadenylation signals by inserting a PCR fragment spanning the entire coding region into the plasmid pART7. The entire regulatory fragment was then removed by digestion with NotI and transferred into the hygromycin resistant binary vector BJ49. The binary vectors were introduced into the *Agrobacterium* strain GV3101, and constructions were checked by re-isolation of the plasmids and restriction enzyme mapping, or by PCR. Transformation to homozygous lec1-1 and lec1-2 mutants were done using the in planta transformation procedure (Bechtold, *et al.*, (1993). *Comptes Rendus de l'Academie des Sciences Serie III Sciences de la Vie*, 316: 1194-1199. Dry seeds from lec1 mutants were selected for transformants by their ability to germinate after desiccation on plates containing 5g/ml hygromycin. The transformed plants

were tested for the present of the transgene by PCR and by screening the siliques for the present of viable seeds.

In Situ Hybridization

Experiments were performed as described previously by Dietrich *et al.* (1989). Plant Cell 1: 73-80. Sections were hybridized with LEC1 antisense probe. As a negative control, the LEC1 antisense probe was hybridized to seed sections of lec1 mutants. In addition, a sense probe was prepared and reacted with the wild type seed sections.

Results

Genetic Interaction Between Leafy Cotyledon-Type Mutants and *abi3*

In order to understand the genetic pathways which regulate late embryogenesis we took advantage of three Arabidopsis mutants *lec2, fus3-3* and *abi3-3* that cause similar defects in late embryogenesis to those of *lec1-1* or *lec1-2*. These mutants are desiccation intolerant, sometimes viviparous and have activated shoot apical meristems. The *lec2* and *fus3-3* mutants are sensitive to ABA and possess trichomes on their cotyledons and therefore can be categorized as leafy cotyledon-type mutants (Meinke *et al.*, 1994). The *abi3-3* mutants belong to a different class of late embryo defective mutations that is insensitive to ABA and does not have trichomes on the cotyledons

The two classes of mutants were crossed to *lec1-1* and *lec1-2* mutants to construct plants homozygous to both mutations. The *lec1* and lec2 mutations interact synergistically, resulting in a double mutant which is arrested in a stage similar to the late heart stage, the double mutant embryo, however, is larger. The *lec1* or *lec2* and *fus3-3* double mutants did not display any epistasis and the resulting embryo had an intermediate phenotype. The *lec1/abi3-3* double mutants and *lec2/abi3-3* double mutants were ABA insensitive and had a lec-like phenotype. There was no different between double mutants that consist of either *lec1-1* or *lec1-2*.

No epistasis was seen between the double mutants indicating that each of the above genes, the LEC-type and ABI3 genes, operate in different genetic pathways.

LEC1 Functions Early in Embryogenesis

The effects of lec1 is not limited to late embryogenesis, it also has a role in early embryogenesis. The embryos of the lec1/lec2 double mutants were arrested in the early

stages of development, while the single mutants developed into mature embryos, suggesting that these genes act early during development.

Further examination of the early stages of the single and double mutations showed defects in the shape, size and cell division pattern of the mutants suspensors. The suspensor of wild type embryo consists of a single file of six to eight cells, whereas the suspensors of the mutants are often enlarged and undergo periclinal divisions. Leafy cotyledon mutants exhibit suspensor anomalies at the globular or transition stage whereas wild type and abi3 mutant do not show any abnormalities.

The number of anomalous suspensors increases as the embryos continue to develop. At the torpedo stage, the wild type suspensor cells undergo programmed cell death, but in the mutants secondary embryos often develop from the abnormal suspensors and, when rescued, give rise to twins.

The Organization of the *LEC1* Locus in Wild Type Plants and *lec1* Mutants

Two mutant alleles of the LEC1 gene have been reported, *lec1-1* and *lec1-2* (Meinke, 1992; West et al., 1994). Both mutants were derived from a population of plants mutagenized insertionally with T-DNA (Feldmann and Marks, 1987), although *lec1-1* is not tagged. The *lec1-2* mutant contains multiple T-DNA insertions. A specific subset of T-DNA fragments were found to be closely linked with the mutation. A genomic library of *lec1-2* was screened using right and left borders T-DNA as probes Genomic clones containing T-DNA fragments that cosegragate with the mutation were isolated and tested on southern blots of both wild type and *lec1-1* plants. Only one clone hybridized with *Arabidopsis* DNA and also gave polymorphic restriction fragment in *lec1-1*.

The *lec1-1* polymorphism resulted from a small deletion, approximately 2 kb in length. Using sequences from the plant fragment flanking the T-DNA, the genomic wild type DNA clones and the *lec1-1* genomic clones were isolated. An EcoRI fragment of 7.4 kb of the genomic wild type DNA that corresponded to the polymorphic restriction fragment in lec1-1 was further analyzed and sequenced. The exact site of the deletion in *lec1-1* was identified using a PCR fragment that was generated by primers, within the expected borders of the deleted fragment, and sequencing.

In the wild type genomic DNA that corresponded to the *lec1-1* deletion, a 626 bp ORF was identified. Southern analysis of wild type DNA and the two mutants DNA probed with the short DNA fragment of the ORF revealed that both the wild type and *lec1-2*

DNA contain the ORF while the *lec1-1* genomic DNA did not hybridize. The exact insertion site of the T-DNA in *lec1-2* mutant was determined by PCR and sequencing and it was found that the T-DNA was inserted 115 bp upstream of the ORF's translational initiation codon in the 5' region of the gene.

At the site of the T-DNA insertion a small deletion of 21 plant nucleic acids and addition of 20 unknown nucleic acids occurred. These results suggest that in *lec1-2* the T-DNA interferes with the regulation of the ORF while in *lec1-1* the whole gene is deleted. Thus, both *lec1* alleles contain DNA disruptions at the same locus, confirming the identity of the *LEC1* locus.

The *lec1* Mutants Can Be Complement by Transformation

To prove that the 7.4 kb genomic wild type fragment indeed contained the ORF of the *LEC1* gene, we used a genomic fragment of 3395 bp (SEQ. ID. No. 3) within that fragment to transform homozygous *lec1-1* and *lec1-2* plants. The clone consists of a 3395 bp BstYI restriction fragment containing the gene and the promoter region. The translation start codon (ATG) of the polypeptide is at 1999 and the stop codon is at 2625 (TGA). There are no introns in the gene.

The transformed plants were selected on hygromycin plates and were tested to contain the wild type DNA fragment by PCR analysis. Both transgenic mutants were able to produce viable progeny, that were desiccation tolerant and did not posses trichomes on their cotyledons. We concluded that the 3.4 kb fragment can complement the lec1 mutation and since there is only one ORF in the deleted 2 kb fragment in *lec1-1* we suggest that this ORF corresponds to the *LEC1* gene.

The *LEC1* Gene is a Member of Gene Family

In order to isolate the *LEC1* gene two cDNA libraries of young siliques were screened using the 7.4 kb DNA fragment as a probe. Seventeen clones were isolated and after further analysis and partial sequencing they were all found to be identical to the genomic ORF. The cDNA contains 626 bp ORF specifying 208 amino acid protein (SEQ. ID. Nos. 1 and 2).

The *LEC1* cDNA was used to hybridize a DNA gel blot containing Ws-O genomic DNA digested with three different restriction enzymes. Using low stringency hybridization we found that there is at least one more gene. This confirmed our finding of two more Arabidopsis ESTs that show homology to the *LEC1* gene.

The *LEC1* gene is Embryo Specific

The *lec1* mutants are affected mostly during embryogenesis. Rescued mutants can give rise to homozygous plants that have no obvious abnormalities other than the presence of trichomes on their cotyledons and their production of defective progeny. Therefore, we expected the *LEC1* gene to have a role mainly during embryogenesis and not during vegetative growth. To test this assumption Poly (A)+ RNA was isolated from siliques, seedling, roots, leaves, stems and buds of wild type plants and from siliques of lec1 plants. Only one band was detected on northern blots using either the *LEC1* gene as a probe or the 7.4 kb genomic DNA fragment suggesting that there is only one gene in the genomic DNA fragment which is active transcriptionally. The transcript was detected only in siliques containing young and mature embryos and was not detected in seedlings, roots, leaves, stems and buds indicating that the *LEC1* gene is indeed embryo specific. In addition, no RNA was detected in siliques of both alleles of lec1 mutants confirming that this ORF corresponds to the LEC1 gene.

Expression Pattern of the LEC1 Gene

To study how the *LEC1* gene specifies cotyledons identity, we analyzed its expression by in situ hybridization. We specifically focused on young developing embryos since the mutants abnormal suspensors phenotype indicates that the *LEC1* gene should be active very early during development.

During embryogenesis, the *LEC1* transcript was first detected in proglobular embryos. The transcript was found in all cells of the proembryo and was also found in the suspensor and the endosperm. However, from the globular stage and on it accumulates more in the outer layer of the embryo, namely the protoderm and in the outer part of the ground meristem leaving the procambium without a signal. At the torpedo stage the signal was stronger in the cotyledons and the root meristem, and was more limited to the protoderm layer. At the bent cotyledon stage the signal was present throughout the embryo and at the last stage of development when the embryo is mature and filling the whole seed we could not detect the *LEC1* transcript. This might be due to sensitivity limitation and may imply that if the *LEC1* transcript is expressed at that stage it is not localized in the mature embryo, but rather spread throughout the embryo.

The *LEC1* gene encodes a Homolog of CCAAT binding factor.

Comparison of the deduced amino acid sequence of LEC1 to the GeneBank reveals significant similarity to a subunit of a transcription factor, the CCAAT box binding factor (CBF). CBFs are highly conserved family of transcription factors that regulate gene activity in eukaryotic organisms Mantvani, *et al.*, . (1992). *Nucl. Acids Res.* 20: 1087-1091. They are hetero-oligomeric proteins that consist of between three to four non-homologous subunits. LEC1 was found to have high similarity to CBF-A subunit. This subunit has three domains; A and C which show no conservation between kingdoms and a central domain, B, which is highly conserved evolutionary. Similarly the *LEC1* gene is composed of three domains. The *LEC1* B domain shares between 75%-85% similarity and 55%-63% identity with different B domains that are found in organisms ranging from yeast to human. Within this central domain, two highly conserved amino acid segments are present. Deletion and mutagenesis analysis in the CBF-A yeast homolog hap3 protein demonstrated that a short region of seven residues (42-48) (LPIANVA) is required for binding the CCAAT box, while the subunit interaction domain lies in the region between residues 69-80 (MQECVSEFISFV) (Xing *et al., supra*). LEC1 protein shares high homology to those regions.

DISCUSSION

The *lec1* mutant belongs to the leafy cotyledon class that interferes mainly with the embryo program and therefore is thought to play a central regulatory role during embryo development. It was shown before that *LEC1* gene activity is required to suppress germination during the maturation stage. Therefore, we analyzed the genetic interaction of homozygous double mutants of the different members of the leafy cotyledon class and the *abi3* mutant that has an important role during embryo maturation. All the five different combinations of the double mutants showed either an intermediate phenotype or an additive effect. No epistatic relationship among the four genes was found. These findings suggest that the different genes act in parallel genetic pathways. Of special interest was the double mutant *lec1/lec2* that was arrested morphologically at the heart stage, but continued to grow in that shape. This double mutant phenotype indicates that both genes *LEC1* and *LEC2* are essential for early morphogenesis and their products may interact directly or indirectly in the young developing embryo.

The Role of *LEC1* in Embryogenesis

One of the proteins that mediate CCAAT box function, is an heteromeric protein called CBF (also called NFY or CP1). CBF is a transcription activator that regulates constitutively expressed genes, but also participates in differential activation of developmental genes Wingender, E. (1993). *Gene Regulation in Eukaryotes* (New York: VCH Publishers). In mammalian cells, three subunits have been identified CBF-A, CBF-B and CBF-C and all of which are required for DNA binding. In yeast, the CBF homolog HAP activates the CYC1 and other genes involved in the mitochondrial electron transport Johnson, *et al.*, Proteins. *Annu. Rev. Biochem.* 58, 799-840. (1989). HAP consists of four subunits hap2, hap3, hap4 and hap5. Only hap2, 3 and 5 are required for DNA binding. CBF-A, B and C show high similarity to the yeast hap3, 2 and 5, respectively. It was also reported that mammalian CBF-A and B can be functionally interchangeable with the corresponding yeast subunits (Sinha *et al.*, *supra.*).

The *LEC1* gene encodes a protein that shows more then 75% similarity to the conserved region of CBF-A. CCAAT motifs are not common in plants' promoters and their role in transcription regulation is not clear. However, maize and *Brassica* homologs have been identified Search in the Arabidopsis GeneBank revealed several ESTs that show high similarity to CBF-A, B and C. Accession numbers of CBF-A (HAP3) homologs: H37368, H76589; CBF-B (HAP2) homologs: T20769; CBF-C (HAP5) homologs: T43909, T44300. These findings and the pleiotropic affects of LEC1 suggest that LEC1 is a member of a heteromeric complex that functions as a transcription factor.

The model suggests that LEC1 acts as transcription activator to several sets of genes, which keep the embryonic program on and repress the germination process. Defective *LEC1* expression partially shuts down the embryonic program and as a result the cotyledons lose their embryonic characteristics and the germination program is active in the embryo.

Example 2

This example demonstrates that *LEC1* is sufficient to induce embryonic pathways in transgenic plants.

The phenotype of *lec1* mutants and the gene's expression pattern indicated that *LEC1* functions specifically during embryogenesis. A *LEC1* cDNA clone under the

control of the cauliflower mosaic virus 35S promoter was transferred into *lec1-1* mutant plants *in planta* using standard methods as described above.

Viable dry seeds were obtained from *lec1-1* mutants transformed with the 35S/*LEC1* construct. However, the transformation efficiency was only approximately 0.6% of that obtained normally. In several experiments, half the seeds that germinated (12/23) produced seedlings with an abnormal morphology. Unlike wild type seedlings, these 35S/*LEC1* seedlings possessed cotyledons that remained fleshy and that failed to expand. Roots often did not extend or extended abnormally and sometimes greened. These seedlings occasionally produced a single pair of organs on the shoot apex at the position normally occupied by leaves. Unlike wild type leaves, these organs did not expand and did not possess trichomes. Morphologically, these leaf-like structures more closely resembled embryonic cotyledons than leaves.

The other 35S/*LEC1* seeds that remained viable after drying produced plants that grow vegetatively. The majority of these plants (7) flowered and produced 100% *lec1* mutant seeds. Amplification experiments confirmed that the seedlings contained the transgene, suggesting that the 35S/*LEC1* gene was inactive in these T2 seeds. No vegetative abnormalities were observed in these plants with the exception that a few displayed defects in apical dominance. A few plants (2) were male sterile and did not produce progeny. One plant that produced progeny segregated 25% mutant Lec1 seeds that, when germinated before desiccation and grown to maturity, gave rise to 100% mutant seed, as expected for a single transgene locus. The other 75% of seeds contained embryos with either a wild type phenotype or a phenotype intermediate between *lec1* mutants and wild type. Only 25% of the dry seed from this plant germinated, and all seedlings resembled the embryo-like seedlings described above. Some seedlings continued to grow and displayed a striking phenotype. These 35S/*LEC1* plants developed two types of structures on leaves. One type resembled embryonic cotyledons while the other looked like intact torpedo stage embryos. Thus, ectopic expression of LEC1 induces the morphogenesis phase of embryo development in vegetative cells.

Because many 35S/*LEC1* seedlings exhibited embryonic characteristics, the seedlings were analyzed for expression of genes specifically active in embryos. Cruciferin A storage protein mRNA accumulated throughout the 35S/*LEC1* seedlings, including the leaf-like structures. Proteins with sizes characteristic of 12S storage protein cruciferin

accumulated in these transgenic seedlings. Thus, *35S/LEC1* seedings displaying an embryo-like phenotype accumulated embryo-specific mRNAs and proteins. *LEC1* mRNA accumulated to a high level in these 35S/*LEC1* seedlings in a pattern similar to early stage embryos but not in wild type seedlings. LEC1 is therefore sufficient to alter the fate of vegetative cells by inducing embryonic programs of development.

The ability of LEC1 to induce embryonic programs of development in vegetative cells establishes the gene as a central regulator of embryogenesis. LEC1 is sufficient to induce both the seed maturation pathway as indicated by the induction of storage protein genes in the *35S/LEC1* seedlings. The presence of ectopic embryos on leaf surfaces and cotyledons at the position of leaves also shows that LEC1 can activate the embryo morphogenesis pathway. Thus, LEC1 regulates both early and late embryonic processes.

The above examples are provided to illustrate the invention but not to limit its scope. Other variants of the invention will be readily apparent to one of ordinary skill in the art and are encompassed by the appended claims. All publications, patents, and patent applications cited herein are hereby incorporated by reference.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: The Regents of the University of California

(ii) TITLE OF INVENTION: Leafy Cotyledon1 Genes and Their Uses

(iii) NUMBER OF SEQUENCES: 18

(iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: Fulbright & Jaworski, LLP
(B) STREET: 865 S. Figueroa Street, 29th Floor
(C) CITY: Los Angeles
(D) STATE: California
(E) COUNTRY: USA
(F) ZIP: 90017-2571

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.30

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER:
(B) FILING DATE:
(C) CLASSIFICATION:

(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: Berliner, Robert
(B) REGISTRATION NUMBER: 20,121
(C) REFERENCE/DOCKET NUMBER: 5555-470

(ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: (213) 892-9200
(B) TELEFAX: (213) 680-4518

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 627 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..627

(D) OTHER INFORMATION: /product= "LEC1"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

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ATC GTG GTC CAG CAG CAA CCA CCA TGT GTG GCT CGT GAG CAA GAC CAA          96
Ile Val Val Gln Gln Gln Pro Pro Cys Val Ala Arg Glu Gln Asp Gln
            20                  25                  30

TAC ATG CCA ATC GCA AAC GTC ATA AGA ATC ATG CGT AAA ACC TTA CCG         144
Tyr Met Pro Ile Ala Asn Val Ile Arg Ile Met Arg Lys Thr Leu Pro
        35                  40                  45

TCT CAC GCC AAA ATC TCT GAC GAC GCC AAA GAA ACG ATT CAA GAA TGT         192
Ser His Ala Lys Ile Ser Asp Asp Ala Lys Glu Thr Ile Gln Glu Cys
    50                  55                  60

GTC TCC GAG TAC ATC AGC TTC GTG ACC GGT GAA GCC AAC GAG CGT TGC         240
Val Ser Glu Tyr Ile Ser Phe Val Thr Gly Glu Ala Asn Glu Arg Cys
65                  70                  75                  80

CAA CGT GAG CAA CGT AAG ACC ATA ACT GCT GAA GAT ATC CTT TGG GCT         288
Gln Arg Glu Gln Arg Lys Thr Ile Thr Ala Glu Asp Ile Leu Trp Ala
                85                  90                  95

ATG AGC AAG CTT GGG TTC GAT AAC TAC GTG GAC CCC CTC ACC GTG TTC         336
Met Ser Lys Leu Gly Phe Asp Asn Tyr Val Asp Pro Leu Thr Val Phe
            100                 105                 110

ATT AAC CGG TAC CGT GAG ATA GAG ACC GAT CGT GGT TCT GCA CTT AGA         384
Ile Asn Arg Tyr Arg Glu Ile Glu Thr Asp Arg Gly Ser Ala Leu Arg
        115                 120                 125

GGT GAG CCA CCG TCG TTG AGA CAA ACC TAT GGA GGA AAT GGT ATT GGG         432
Gly Glu Pro Pro Ser Leu Arg Gln Thr Tyr Gly Gly Asn Gly Ile Gly
    130                 135                 140

TTT CAC GGC CCA TCT CAT GGC CTA CCT CCT CCG GGT CCT TAT GGT TAT         480
Phe His Gly Pro Ser His Gly Leu Pro Pro Pro Gly Pro Tyr Gly Tyr
145                 150                 155                 160

GGT ATG TTG GAC CAA TCC ATG GTT ATG GGA GGT GGT CGG TAC TAC CAA         528
Gly Met Leu Asp Gln Ser Met Val Met Gly Gly Gly Arg Tyr Tyr Gln
                165                 170                 175

AAC GGG TCG TCG GGT CAA GAT GAA TCC AGT GTT GGT GGT GGC TCT TCG         576
Asn Gly Ser Ser Gly Gln Asp Glu Ser Ser Val Gly Gly Gly Ser Ser
            180                 185                 190

TCT TCC ATT AAC GGA ATG CCG GCT TTT GAC CAT TAT GGT CAG TAT AAG         624
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TGA                                                                     627
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(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 208 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

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    130                 135                 140

Phe His Gly Pro Ser His Gly Leu Pro Pro Pro Gly Pro Tyr Gly Tyr
145                 150                 155                 160

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                165                 170                 175

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Ser Ser Ile Asn Gly Met Pro Ala Phe Asp His Tyr Gly Gln Tyr Lys
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(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 3395 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

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CACCAATATC TTGTACTAAC CTTTTGTAAT TTTCCTATAA ACATTTTAAA AGGCTAATTT 240

AAATAAAAAT TACAATAAAC GTGATAACTC ACTTTCGTAA CGCATATTTA TTCAAATATA 300

CCAAAATTTA CCATTTTAAG TAAGAGAATC TTTTTAAAAT TAATTTTCAA TTTCATTAAT 360

TAAGAAACAA AGAATTTACT GAAACCTATA TTTTATTAAA TTTTAATAAA ATATATGACT 420

AAAATAACGT CACGTGAATC TTTCTCAGCC GTTCGATAAT CGAATACTTT ATTGACTAAG 480

TATTTATTTA GAAAATTTTA AACAACACTT AATTTCTAGA AACAAAGAGA GCCTCATATG 540

TATAAAAATC TTCTTCTTAT CTTTCTTTCT TTCTTAATAG TCTTTATTTT TACTTAATTA 600

CTTTGGTAAT TTGTGAAAAA CACAACCAAT GAGAGAAGAG CAGTTTGACT GGCCACATAG 660

CCAATGAGAC AAGCCAATGG GAAAGAGATA TAGAGACCTC GTAAGAACCG CTCCTTTGCC 720

ATTTGTATCA TCTCTCTATA AAACCACTCA ACCATCAACC TNTCTTTGCA TGCAACAAAT 780

CACTCAAATA ATTATTTTAT AAAGAACAAA AAAAAAAAGA CGGCAGAGAA ACAATGGAAC 840

GTGGAGCTCC CTTCTCTCAC TATCAGCTAC CCAAATCCAT CTCTGGTAAT CTAAGTGGCT 900

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GAATCTGAAC AGTCTACTCA ACGGGGTCCA TAAGTCTAGA ATGTAGACCC CACAAACTTA 1800

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AGCAACAACC CAACCCCAAT GACCAGCTCA GTCATAGTAG CCGGCGCCGG TGACAAGAAC 2040

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CCAATCGCAA ACGTCATAAG AATCATGCGT AAAACCTTAC CGTCTCACGC CAAAATCTCT 2160

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TGGGCTATGA GCAAGCTTGG GTTCGATAAC TACGTGGACC CCCTCACCGT GTTCATTAAC 2340

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AATAACATTT CTATATAATG TTGCTTCTTT AAGGAAAAGT GTTGTATGTC AATACTTTAT 2760

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TGCAACATTA TATATTTACA TGATCTAAAG GTTTTGTAAT TCAAAAGCTG TCATAGTTAG 2880

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CCAAGCCAAA AGGTTATAAA ATCTAAATTC GTTGAGTTGT CAAACTTCTG AAGATTGCTA 3000

TCCTCTTTGA GTTGCTTTCT TTTGGGTGCT TGAGTTTCAT TAGGCTGAGC TGACTCGTTG 3060

CTCTCTAGTC TTTCATCTCT GTCTTTTCCA AGGATTCATA ACGTTGGTCG CTCTCTGTTT 3120

CTGCCTACAC TTCTTCAAGG GATCATTACT GAGGCTAAGA GTTAAAGACC TGAACCATGG 3180

TTTTCTGTAA CTGGTTCAAG TTCATTCTCC GGTTATTGTG TGGTTATCTT TCGGTTAGAT 3240

TGAAACCCAT ATGTTTGCTC TGTTTCTTCT AGTTCCAAGT TTAATTTCCG GTTATTGTTT 3300

GGCTTTTTAA AAGTTTTTAA GGTCTATTCT ATGTAAAGAC TATTCTACGT ACGTACATTT 3360

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(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 7560 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

AATTNACCCT CACTAAAGGG AACAAAAGCT GGGTACCGGG CCCCCCCTCG AGGTCGACGG 60

TATCGATAAG CTTGATATCG AATTCGTGGC CATTAGACCC ATAACTATAT GACGATGTTA 120

AAGAGAAAAT AAATCATAAA TAAAATAAGA GTCCTTATCA ATAAACCTAA TTGGCTAATT 180

TCAACCTCAA AGAGTAGTAG GAACAGGTAA GGTGAAGCCA AACAGCTCCT TTTACAGTTG 240

GACCACTAGA GCTGATCTGG CATACAAAGT ATGCTTATTG GGCTGTCACG GCCCATCCGC 300

AAAATGTCGT TGGTTACGAA GCATCCACGA CATAGACGGT GCCACATGTT AGAAAAGTGT 360

TTCGGCGATC AAGATTGTGT CCACATCATT AGACGTCTGA ACTGTCCACG TGTCTATCAA 420

AGCTGGCGTC AAACATTACG TTTTCGTCGT TTGCGCCTCC TAGTTCACAC GTGCAACGAA 480

CGCGTGCGAC GTATCAAAAT TGTTAATTTT AGCCATGTAT AAAGAATATC TACAAAATTA 540

ACCTCAGGAA TATTTTTGTT TTTTCAATTG AGGCCATAAT ATACNTNCCG ATNGAAAAAT 600

TTTNCANCAT ATCNCTAATA TCAAAAAATT ATGATGTTAG TAAACGTAAA AAATTTACAC 660

AAAATAANTT TCACAAAACT TANNGGGGAA ATTGGAACAA ANAAAAGACT GGTGAGTGAT 720

AAGCGATGAT GGCCGGTGAA TCAGGTAGCC GTCCTACAAC GTGGTTGATT TTGAGCAAAC 780

TCCTATCTAC TCTTCACACT ATTGGAAATC CCAAAATGTC GTCACACCAT AATAATGTGA 840

ATTTTGTTAT GGAATTTGAG GGAAACAGTA GATATATGTT TCAACCAGTG AAAGTTACCC 900

TCCTTTGGAC ATATCTACGA NAGTAGAAAG TAGAAACATT CACTAAACGT GACAACTTTA 960

TAAATTTTCT TTTTGTAACT TTTCTTTAGA TTTATTTACG ANAAGAGAAA TATAAACGTC 1020

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GTTTTTTGTT TATCTCTTCT CTCATAGGCA TGTCCACAAA AGGGTAAGTT TTACTGGTTC 1140

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TTACCAGAAG TCATATTTCC TTAACCTCAG AATAAGTAAA TCTTCTAGTT TATTATTTGA 2040

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TGTATTATCC TATCTCTTTA GCTATCCTTT CCTTGCTCTT GCTCCACCTG CATGTGGCCT 2160

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TTTTAAAATT AATTTTCAAT TTCATTAATT AAGAAACAAA GAATTTACTG AAACCTATAT 2820

TTTATTAAAT TTTAATAAAA TATATGACTA AAATAACGTC ACGTGAATCT TTCTCAGCCG 2880

TTCGATAATC GAATACTTTA TTGACTAAGT ATTTATTTAG AAAATTTTAA ACAACACTTA 2940

ATTTCTAGAA ACAAAGAGAG CCTCATATGT ATAAAAATCT TCTTCTTATC TTTCTTTCTT 3000

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GCGATCGTTT TGAGGCGTCC CAATTTTTTT TGTTTCTTTT TATATGAGAA ATGGGTTTAA 7440

GAAAAACTGC AATTTTGTCC ATAAAGCTAG TCAGAATTCC TGCAGCCCGG GGGATCCACT 7500

AGTTCTAGAG CGGCCGCCAC CGCGGTGGAG CTCCAATTCG CCCTATAGTG AGTCGTATTA 7560

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 7 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

Met Pro Ile Ala Asn Val Ile
1 5

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 12 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

Ile Gln Glu Cys Val Ser Glu Tyr Ile Ser Phe Val
1 5 10

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 25 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

GGAATTCAGC AACAACCCAA CCCCA 25

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

GCTCTAGACA TACAACACTT TTCCTTA 27

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

ATGACCAGCT CAGTCATAGT AGC 23

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

GCCACACATG GTGGTTGCTG CTG 23

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23 base pairs
(B) TYPE: nucleic acid

(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

GAGATAGAGA CCGATCGTGG TTC 23

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

TCACTTATAC TGACCATAAT GGTC 24

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

GCATAGATGC ACTCGAAATC AGCC 24

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

GCTTGGTAAT AATTGTCATT AG 22

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

CTAAAAACAT CTACGGTTCA 20

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

TTTGTGGTTG ACCGTTTGGC

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 7 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

Leu Pro Ile Ala Asn Val Ala
1 5

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 12 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

Met Gln Glu Cys Val Ser Glu Phe Ile Ser Phe Val
1 5 10

WHAT IS CLAIMED IS:

1. An isolated nucleic acid molecule comprising a *LEC1* polynucleotide sequence, which polynucleotide sequence specifically hybridizes to SEQ. ID. No. 1 under stringent conditions.

2. The isolated nucleic acid molecule of claim 1, wherein the *LEC1* polynucleotide is between about 100 nucleotides and about 630 nucleotides in length.

3. The isolated nucleic acid molecule of claim 1, wherein the *LEC1* polynucleotide is SEQ. ID. No. 1.

4. The isolated nucleic acid molecule of claim 1, wherein the *LEC1* polynucleotide encodes a LEC1 polypeptide of between about 50 and about 210 amino acids.

5. The isolated nucleic acid molecule of claim 4, wherein the LEC1 polypeptide has an amino acid sequence as shown in SEQ. ID. No. 2.

6. The isolated nucleic acid molecule of claim 1, further comprising a plant promoter operably linked to the *LEC1* polynucleotide.

7. The isolated nucleic acid molecule of claim 6, wherein the plant promoter is from a *LEC1* gene.

8. The isolated nucleic acid of claim 7, wherein the *LEC1* gene is as shown in SEQ. ID. No. 3.

9. The isolated nucleic acid of claim 7, wherein the *LEC1* gene is as shown in SEQ. ID. No. 4.

10. The isolated nucleic acid of claim 7, wherein the *LEC1* polynucleotide is linked to the promoter in an antisense orientation.

11. An isolated nucleic acid molecule comprising a *LEC1* polynucleotide sequence, which polynucleotide sequence encodes LEC1 polypeptide of between about 50 and about 210 amino acids.

12. The isolated nucleic acid of claim 10, wherein the LEC1 polypeptide has an amino acid sequence as shown in SEQ. ID. No. 2.

13. A transgenic plant comprising an expression cassette containing a plant promoter operably linked to a heterologous *LEC1* polynucleotide that specifically hybridizes to SEQ. ID. No. 1 under stringent conditions.

14. The transgenic plant of claim 12, wherein the heterologous *LEC1* polynucleotide encodes a LEC1 polypeptide.

15. The transgenic plant of claim 13, wherein the LEC1 polypeptide is SEQ. ID. No. 2.

16. The transgenic plant of claim 12, wherein the heterologous *LEC1* polynucleotide is linked to the promoter in an antisense orientation.

17. The transgenic plant of claim 12, wherein the plant promoter is from a *LEC1* gene.

18. The transgenic plant of claim 16, wherein the *LEC1* gene is as shown in SEQ. ID. No. 3.

19. The transgenic plant of claim 12, which is a member of the genus *Brassica*.

20. A method of modulating seed development in a plant, the method comprising introducing into the plant an expression cassette containing a plant promoter

operably linked to a heterologous *LEC1* polynucleotide that specifically hybridizes to SEQ. ID. No. 1 under stringent conditions.

21. The method of claim 19, wherein the heterologous *LEC1* polynucleotide encodes a LEC1 polypeptide.

22. The method of claim 20, wherein the LEC1 polypeptide has an amino acid sequence as shown in SEQ. ID. No. 2.

23. The method of claim 19, wherein the heterologous *LEC1* polynucleotide is linked to the promoter in an antisense orientation.

24. The method of claim 19, wherein the heterologous *LEC1* polynucleotide is SEQ. ID. No. 1.

25. The method of claim 19, wherein the plant promoter is from a *LEC1* gene.

26. The method of claim 19, wherein the *LEC1* gene is as shown in SEQ. ID. No. 3.

27. The method of claim 19, wherein the plant is a member of the genus *Brassica*.

28. The method of claim 19, wherein the expression cassette is introduced into the plant through a sexual cross.

29. An isolated nucleic acid molecule comprising a plant promoter that specifically hybridizes to a polynucleotide sequence consisting of nucleotides 1 to 1998 of SEQ. ID. No. 3.

30. The isolated nucleic acid molecule of claim 28, wherein the plant promoter sequence consists essentially of nucleotides 1 to 1998 of SEQ. ID. No. 3.

31. The isolated nucleic acid molecule of claim 28, wherein the plant promoter sequence is a subsequence of SEQ. ID. No. 4.

32. The isolated nucleic acid molecule of claim 28, further comprising a polynucleotide sequence operably linked to the plant promoter sequence.

33. The isolated nucleic acid of claim 30, wherein the polynucleotide sequence operably linked to the plant promoter sequence encodes a desired polypeptide.

34. The isolated nucleic acid molecule of claim 28, wherein the polynucleotide sequence is linked to the promoter in an antisense orientation.

35. A transgenic plant comprising an expression cassette containing a *LEC1* promoter operably linked to a heterologous polynucleotide sequence, wherein the *LEC1* promoter specifically hybridizes to SEQ. ID. No. 3 under stringent conditions.

36. The transgenic plant of claim 33, wherein the polynucleotide sequence encodes a desired polypeptide.

37. The transgenic plant of claim 33, wherein the heterologous polynucleotide sequence is linked to the *LEC1* promoter in an antisense orientation.

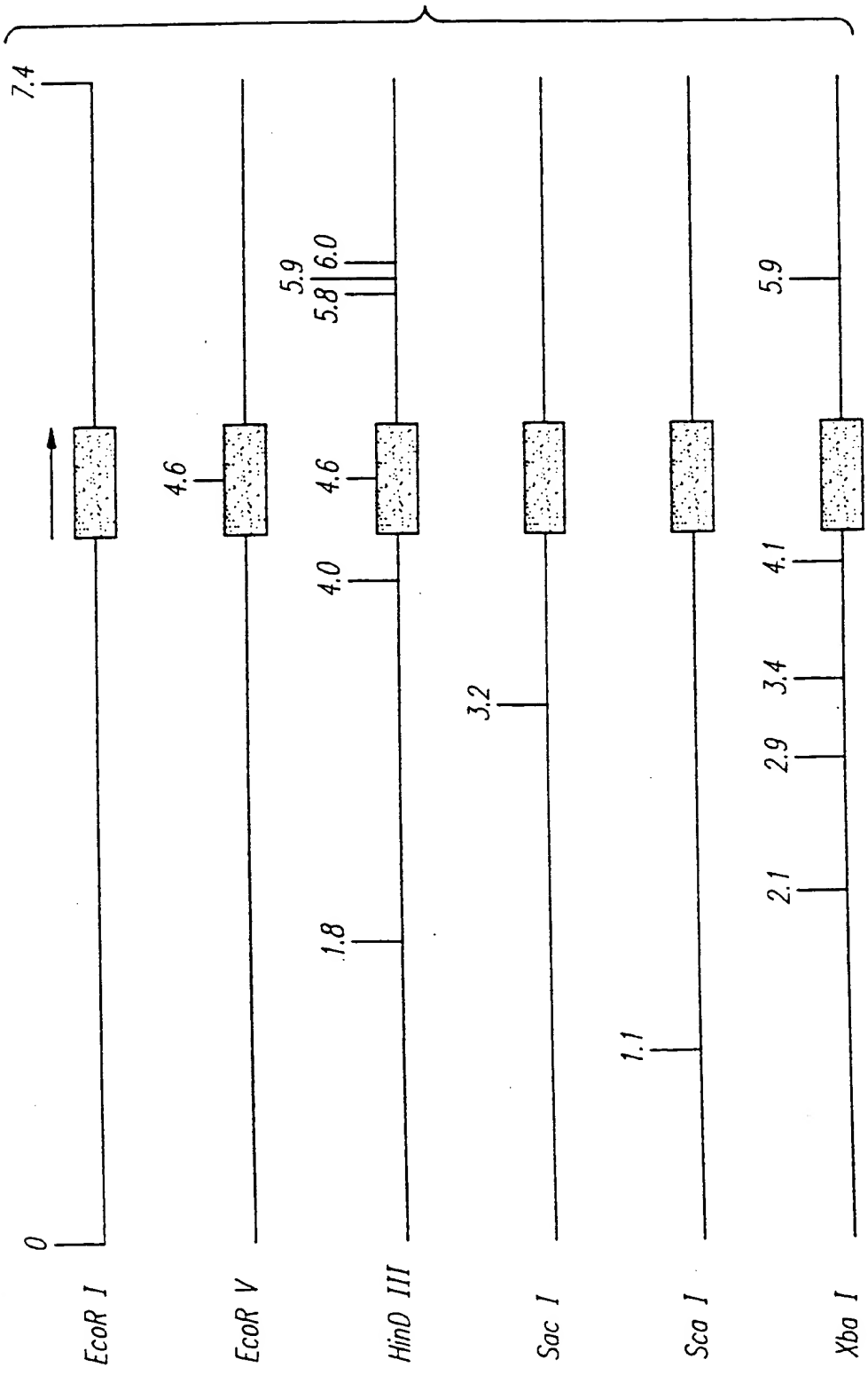
38. The transgenic plant of claim 33, wherein the *LEC1* promoter is as shown in SEQ. ID. No. 3.

39. The transgenic plant of claim 33, which is a member of the genus *Brassica*.

40. A method of targeting expression of a polynucleotide to a seed, the method comprising introducing into a plant an expression cassette containing a *LEC1* promoter operably linked to a heterologous polynucleotide sequence, wherein the *LEC1* promoter specifically hybridizes to a polynucleotide sequence consisting of nucleotides 1 to -1998 of SEQ. ID. No. 3.

41. The method of claim 38, wherein the heterologous polynucleotide sequence encodes a desired polypeptide.

42. The method of claim 38, wherein the heterologous polynucleotide sequence is linked to the promoter in an antisense orientation.


7.4
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4.6
EcoR V
5.9
5.8
6.0
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4.0
1.8
HinD III
3.2
Sac I
1.1
Sca I
5.9
4.1
3.4
2.9
2.1
Xba I

FIG. 1

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US98/02998

A. CLASSIFICATION OF SUBJECT MATTER
IPC(6) : C12N 15/00, 15/09, 15/29, 15/82; A01H 1/00, 3/00, 5/00
US CL : 536/23.6, 24.1, 24.5; 435/172.3, 252.2, 320.1; 800/205
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 536/23.6, 24.1, 24.5; 435/172.3, 252.2, 320.1; 800/205

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Agricola, Wpids, STN, Biosis, NCBI, APS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	MEINKE, D.W. A homeotic Mutant of *Arabidopsis thaliana* with Leafy Cotyledons. Science. 04 December 1992, Vol. 258, pages 1647-1650. See entire reference.	1-42
Y	HECK et al. AGL15, a MADS Domain Protein Expressed in Developing Embryos. The Plant Cell. August 1995, Vol. 7, pages 1271-1282, see entire reference.	1-42
Y	VALVEKENS et al. *Agrobacterium tumefaciens*-Mediated Transformation of *Arabidopsis thaliana* Root Explants by Using Kanamycin Selection. Proc. Natl. Acad. Sci., USA, August 1988, Vol. 85, pages 5536-5540, see entire reference	13-28, 35-42

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"B"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search	Date of mailing of the international search report
06 APRIL 1998	09 JUN 1998
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer Ousama M-Faiz Zaghmout Telephone No. (703) 308-0196

Form PCT/ISA/210 (second sheet)(July 1992)*

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US98/02998

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	CHAN et al. Novel Gene Expression System for Plant Cells Based on Induction of α-Amylase Promoter by Carbohydrate Starvation. The Journal of Biological Chemistry. 01 July 1994, Vol. 269, No. 26, pages 17635-17641, see entire reference.	7-10, 17, 25, 29-42
Y	SHIMADA et al. Antisense Regulation of the Rice Waxy Gene Expression Using a PCR-Amplified Fragment of the Rice Genome Reduces the Amylose Content in Grain Starch. Theor. Appl. Genet. 1993, Vol. 86, pages 665-672, see entire content.	10, 12, 14, 16-19, 21, 23-28, 34, 37, 42

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US98/02998

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

Please See Extra Sheet.

1. ☒ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING
This ISA found multiple inventions as follows:

This application contains the following inventions or groups of inventions which are not linked as to form a single inventive concept under PCT Rule 13.1.

Group I. Claims 1-6, 11, 13, 15, 20, and 22 are drawn to nucleic acid molecule comprising a LEC1 gene, vectors containing it in sense orientation, methods for their use to transform plants, and the resultant transgenic plants, classified in class 800, subclass 205 for example.

Group II. Claims 7-10, 17, 25, 29- 42 are drawn to LEC1 promoters, methods for their use in constructs to transform plants, and the resultant transgenic plants, classified in class 536, subclass 24.1 for example.

Group III. Claims 10, 12, 14, 16-19, 21, 23-28, 34, 37, and 42 are drawn to vectors containing sequences in antisense orientation, methods for their use to transform plants, and the resultant transgenic plants, classified in class 536, subclass 24.5 for example.

The inventions listed as groups I, II and III do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons: The invention of group I, drawn to a first process of making a first product, involves making the construct and transforming plants not required by the groups II and III.

The invention of group I involves the usage of coding sequences of LEC1 in the sense orientation, not required by groups II-III.

The invention of group II involves the LEC1 promoter sequences and their usage not required by groups I and III, and a non-LEC1 structural gene not required by group I.

The invention of group III involves the usage of coding sequences of LEC1 or other genes in the antisense orientation, and methods for evaluation of gene suppression, not required by groups I-II.